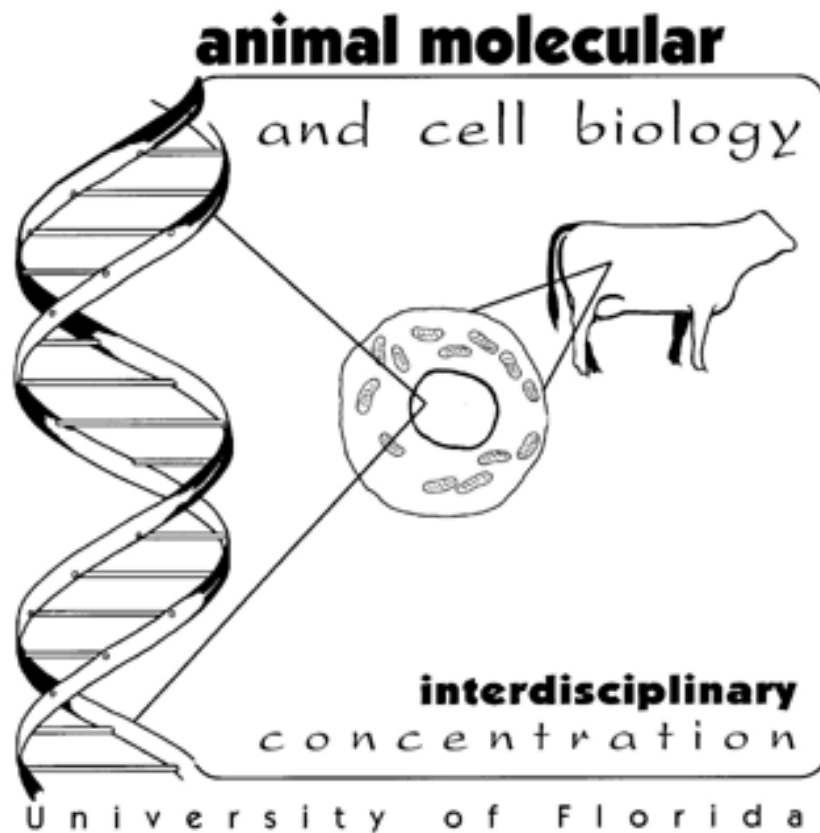


University of Florida

Animal Molecular and Cellular Biology

Second Annual Research Symposium



Chinsegut Hill Conference Center, Brooksville, FL

April 2nd and 3rd, 2004

Animal Molecular and Cellular Biology **Second Annual Research Symposium**

WELCOME

It is our great pleasure to welcome you to the Second Annual Research Symposium for the Animal Molecular and Cellular Biology Interdisciplinary Graduate Program, University of Florida, held at the Chinsegut Hill Conference and Retreat Center. This two-day event has been planned 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 an invited presentation from Dr. Lokenga Badinga, and our Distinguished AMCB Lecturer, Dr. John Dobrinsky, from the United States Department of Agriculture. Additionally, we have planned our Friday evening social events and a barbecue to allow participants time to relax and enjoy the area. We look for this year's symposium to be another great success and hope you find it enlightening and enjoyable.

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

ACKNOWLEDGEMENTS

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

Dr. Richard Jones, Dean of Research, IFAS

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

Dr. Jimmy Cheek, Dean of Academic Programs, IFAS

Dr. Jane Luzar, Associate Dean of Academic Programs, IFAS

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

Chinsegut Hill Conference and Retreat Center



The site now known as Chinsegut Hill was staked from the United States government by Colonel Pearson of Columbia, South Carolina, in 1842. Pearson's youngest daughter, Lydia Florida, later became the wife of Governor Fleming of Florida. Part of the present manor house was built in 1849, with later additions making it the large house it is today. In 1852, Colonel Pearson sold the property to Colonel Francis H. Ederington, and in 1904, Colonel Raymond Robins bought the Hill from the grandchildren of Colonel Ederington, Mallory and Ernest Snow.

Colonel Robins had a colorful career which started when, at the age of ten, he came to Florida from his native Kentucky and lived near Chinsegut Hill on a farm known as Bodine Grove. As a small boy, it became his wish to someday own this hill, then known as "Snow Hill." He left the county at the age of seventeen, worked in coal and lead mines, and went to the Klondike in search of gold. There, as a minister and a social worker, he founded a church and a hospital. When he returned to Florida from the Klondike, it was with sufficient gold to fulfill his boyhood dream of owning the property. Colonel Robins named the property "Chinsegut Hill." The word "Chinsegut" is an Inuit word that means "The Spirit of Lost Things".

Colonel Robins was honored with the title of colonel for his work with the American Red Cross in Russia during the First World War. When the Russian Revolution broke out, he was the sole Allied representative in Russia. Through his work with the Red Cross, he became acquainted with Lenin and Trotsky. In 1918, Mrs. Robins planted an acorn on the Chinsegut grounds as the Colonel met with Lenin in Russia. When Mrs. Robins told the Colonel of the planting, he responded, "If it grows, we will call it the Lenin Oak." The Lenin Oak stands majestically on the grounds today.

In 1932, Colonel and Mrs. Robins gave their property to the Federal Government as a Wildlife Refuge, Forest Preserve and Agricultural Experiment Station. Mrs. Robins died in 1945 and Colonel Robins in 1954. The house was used by the University of Florida until 1958 as a branch library. Today, the house and the ground of Chinsegut Hill are used by the University of South Florida as a conference and retreat center.

(Adapted from http://www.auxsvc.usf.edu/chinsegut_history.html)

SYMPOSIUM AGENDA

Friday, April 2, 2004

9:30AM - Leave Animal Sciences for the Symposium

12:00 PM – Lunch at Chinsegut (Hosted by AMCB Faculty)

1:00 PM - Welcome and Introductory Comments

1:15 PM – Dr. John Dobrinsky, Distinguished AMCB Lecturer

Pig Embryo Cryopreservation: Supercool(ed) Cell Biology

2:15 PM – Break/AMCB Faculty Meeting

3:00 PM - Student Presentations Session 1 – Moderator: Dr. Aydin Guzeloglu

3:00-3:20 Dean Jousan

3:20-3:40 Anne Evans

3:40-4:00 Liz Johnson

4:00-4:20 Flavio Silvestre

4:30 PM – Dr. Lokenga Badinga, invited speaker

Production, Metabolic and Molecular Responses of Periparturient Holstein Cows to Dietary Conjugated Linoleic Acid and Trans-Octadecenoic Acids

5:30 PM – Volleyball and Social

7:00 PM - Barbecue on the Ranch (Hosted by AMCB Faculty)

Saturday, April 3, 2004

7:30AM – Breakfast (Hosted by AMCB Faculty)

8:30 AM - Student Presentations Session 2 – Moderator: Mr. F. Dean Jousan

8:30-8:50 Lynda Miller

8:50-9:10 Sara Wagner

9:10-9:30 Maria Padua

9:30-9:50 Stacey Goicoa

9:50-10:10 Todd Bilby

10:10 AM - Break

10:30-10:50 Jeremy Block – Moderator: Ms. Tricia Schnulle

10:50-11:10 Cristina Caldari-Torres

11:10-11:30 Dr. Zvi Roth

11:30-11:50 Luiz Augusto de Castro e Paula

11:50-12:10 Carlos Rodriguez-Sallaberry

12:10-12:30 Amber Brad

12:30 AM - Wrap Up/Critique

12:45 AM – Adjourn

INVITED SPEAKERS

2004 Distinguished AMCB Lecturer, Dr. John Dobrinsky, United States Department of Agriculture, Agricultural Research Service, Biotechnology and Germplasm Laboratory

Pig Embryo Cryopreservation: Supercool(ed) Cell Biology

John Dobrinsky is a Research Physiologist specializing in domestic animal embryology for the US Department of Agriculture, Agricultural Research Service, in Beltsville, Maryland. His research program investigates embryonic development and avenues for germplasm conservation in livestock and has led to the development of six major breakthroughs in swine embryology, including: the successful cryopreservation of swine embryos by cytoskeletal stabilization and vitrification which produced the first live offspring ever after warming and subsequent transfer of cryopreserved embryos. This work culminated in patenting of the USDA Swine Embryo Cryopreservation Technology. By invitation to cooperate in the laboratory of Professor Ian Wilmut, Roslin Institute, Scotland, John co-developed somatic cell nuclear transfer technology for production of cloned pigs. This technology was used by the Roslin Institute to make Dolly, the first cloned mammal ever. It was adapted for cloning pigs to be used in agricultural production and biomedical research. John also developed the first defined swine embryo culture medium and was a co-investigator of an international team that produced the first sex-preselected offspring obtained from in vitro fertilization in swine, utilizing the patented Beltsville Sperm Sexing Technology. John is also co-investigator in an ARS team that is developing Serial Analysis of Gene Expression (SAGE) technology for studying developmental biology of swine embryos. It is hoped that this technology will provide valuable insight into preventing early embryonic loss and maintaining pregnancy in swine. Most recently, John was a co-investigator in of a novel attempt to generate progeny by intracytoplasmic sperm injection with boar sperm obtained from ectopic xenografts of neonatal pig testes under the back skin of immunocompromised mice. While this technology works in xenografts from mice to mice, we developed this technology to show that cross-species spermatogenesis between phylogenetically distant species like pigs and mice is possible, providing a novel opportunity for the preservation of genetic material from sexually immature and mature individuals. Birth of normal piglets from this technology will have far reaching implications for conservation of endangered species or valuable livestock that may die before they reach puberty, and provide the preservation of future fertility in childhood cancer patients who may have to undergo chemotherapy or radiation therapy.

Contact information:

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AMCB Invited Speaker, Dr. Lokenga Badinga, UF Department of Animal Sciences, AMCB Faculty

Production, Metabolic and Molecular Responses of Periparturient Holstein Cows to Dietary Conjugated Linoleic Acid and Trans-Octadecenoic Acids

Dr. Lokenga Badinga, received his M.S. in Dairy Sciences and his Ph.D. in Animal Sciences at the University of Florida and is currently an Assistant Professor of Nutritional Physiology in the Department of Animal Sciences. Dr. Badinga's research interests include the investigation of cellular and molecular mechanisms by which dietary factors control lipid and glucose metabolism in dairy cattle, identification of dietary components which minimize metabolic upsets and increase milk production in transition dairy cattle, and to study the effects of supplemental fats on reproductive efficiency in postpartum dairy cattle.

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ABSTRACTS

INSULIN-LIKE GROWTH FACTOR-1 AS A SURVIVAL FACTOR FOR BOVINE EMBRYOS SUBJECTED TO HEAT SHOCK

F.D. Jousan* and P.J. Hansen

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Insulin-like growth factor-1 (IGF-1) is a survival factor that modifies the response of a cell to injury. Here, it was hypothesized that the thermoprotective effects of IGF-1 would reduce apoptosis and subsequently allow heat-shocked embryos to proceed through development to the blastocyst stage. Embryos were produced in vitro and cultured in KSOM \pm 100 ng mL⁻¹ IGF-1. In Exp. 1, embryos at 5 days post-insemination (dpi) having = 16 cells were harvested (7 replicates; 86-100 embryos/treatment), placed in fresh drops containing the same IGF-1 treatment as received from day 0-5, and cultured at either 38.5°C for 24 h or 41°C for 9 h and 38.5°C for 15 h. At 38.5°C, IGF-1 did not affect total cell number (60.5 vs 64.4 for control and IGF-1, respectively; SEM = 2.3) or percent of blastomeres undergoing apoptosis as determined by TUNEL (5.9% vs 5.7%; SEM = 0.6%). Heat shock reduced total cell number ($P < 0.05$) and increased the percent of cells that were TUNEL-positive ($P < 0.001$). For heat-shocked embryos, total cell number was 46.0 for control vs 59.8 for IGF-1 (SEM = 2.3) and percent blastomeres that were TUNEL-positive was 11.6% for control vs 5.9% for IGF-1 (SEM = 0.6%). Effects of heat shock were less for IGF-1 treated embryos (temperature x IGF-1, $P = 0.07$ for cell number and $P < 0.01$ for TUNEL). For Exp. 2, embryos at 5 dpi having = 16 cells were harvested (11 replicates; 191-236 embryos/treatment), placed in fresh drops containing the same IGF-1 treatment as received from day 0-5, and exposed to either 38.5°C continuously or 41°C for 9 h followed by 38.5°C until 8 dpi. Blastocysts were then fixed to determine the number of total and apoptotic nuclei using the TUNEL assay. More embryos cultured in IGF-1 became blastocysts than control embryos ($P < 0.001$). This was true for embryos at 38.5°C (64.1% vs 43.4%) and for embryos exposed to 41°C (50.2% vs 24.5%; SEM = 2.6%). Heat shock also reduced blastocyst development ($P < 0.001$). The reduction in blastocyst development caused by 41°C as a percentage of development at 38.5°C was less ($P < 0.05$) for IGF-1 treated embryos than for control embryos (19.4% vs 46.4% decrease; SEM = 3.8%). Blastocyst cell number was affected by IGF-1 ($P < 0.001$) and heat shock ($P < 0.01$). Least-squares means at 38.5°C were 147.5 vs 135.5 for IGF-1 treated and control embryos while least-squares means at 41°C were 133.6 vs 104.3 for IGF-1 treated and control embryos (SEM = 8.4). The proportion of blastomeres that were TUNEL-positive was affected by IGF-1 ($P < 0.001$), heat shock ($P < 0.01$), and IGF-1 x heat shock ($P < 0.10$). In particular, heat shock increased the percentage of blastomeres that were TUNEL-positive and this increase tended to be less for IGF-1 treated embryos (6.5% and 7.5% for 38.5°C and 41°C) than for control embryos (8.0% and 11.5% for 38.5°C and 41°C; SEM = 0.8%). Thus, IGF-1 serves as a survival factor for the developing embryo exposed to heat shock by allowing development to the blastocyst stage and by avoiding the increase in blastocyst apoptosis caused by heat shock. (Support: USDA NRICGP 2002-35203-12664 and USDA IFAFS #2001-11318).

IMPROVING PREIMPLANTATION GENETIC DIAGNOSIS IN LIVESTOCK

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The introduction of artificial reproductive technologies, such as in-vitro fertilization (IVF) and cloning, into the livestock industry has been beneficial for producing offspring with desired genotypes. However, these techniques are not yet economically feasible for the producer due to high costs associated with abortions and recipient maintenance. One cause of early embryonic death and abortion is genetic abnormalities. Fortunately, advances in DNA technologies have given the livestock industries the opportunity to perform genetic screening prior to embryo transfer by using a technique called preimplantation genetic diagnosis (PGD). A single blastomere is removed from a day 3 embryo and subjected to polymerase chain reaction (PCR), karyotyping, or fluorescent in-situ hybridization (FISH). The genetic diagnosis is made before day 7, the time embryo transfer would be performed. However, there are specific problems with PGD. Karyotyping individual interphase blastomeres has been inefficient since karyotyping can only be done with cells in metaphase. Several studies in the mouse and human have shown that it is possible to inject a blastomere into an enucleated oocyte to induce chromosome condensation, thus allowing production of metaphase chromosome spreads. Unfortunately, this technique has been inefficient because the fusion rate of the blastomere into the enucleated egg is low (50-70%). Furthermore, of the blastomeres that fuse, no more than 50% produce analyzable karyotypes. The hypothesis of this project is that PGD will be enhanced through improved nuclear introduction and chromatin condensation techniques. The first objective of this study is to improve the efficiency of PGD by using a piezo device to introduce blastomere nuclei into enucleated oocytes, bypassing cell fusion. The piezo device, which has been used successfully for somatic cell nuclear transfer in mice, will be used to inject the blastomere nuclei directly into the cytoplasm of the oocyte. It is expected that this will achieve near 100% efficiency of delivering the blastomere nuclei into the egg cytoplasm, which is required for chromatin condensation to occur. The next objective of this study is to improve the efficiency of inducing chromatin condensation using phosphatase inhibitors. This study will improve existing protocols for karyotyping and fluorescent in-situ hybridization (FISH) using the protein phosphatase inhibitors colcemid, calyculin A, cantharidin, and fostriecin to induce prechromosome condensation. Optimal concentrations and incubation times will be elucidated for each chemical. It is expected that the phosphatase inhibitors will induce chromosome condensation in the blastomeres or couplets and enhance production of analyzable metaphase spreads. The success of this study has major implications in the livestock industry for either selecting against genetic defects or selection for economically beneficial traits prior to embryo transfer. By developing an efficient PGD protocol, early embryonic death and abortion in livestock can be reduced, making artificial reproductive technologies more economically feasible for livestock producers. (Supported by UF Opportunity Grant)

TRANS-10,CIS-12 CONJUGATED LINOLEIC ACID UP-REGULATES ACYL COA OXIDASE, 3-HYDROXY, 3-METHYLGLUTARYL COA REDUCTASE AND APOLIPOPROTEIN A-I GENE EXPRESSION IN HUMAN AND RAT HEPATOMA CELL LINES

E.S. Johnson* and L. Badinga

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Conjugated linoleic acids (CLA) refer to a group of polyunsaturated fatty acids (PUFA) that exist as positional and geometric isomers of octadecadienoates (C18:2). Molecular actions of CLA appear to include modulation of eicosanoid synthesis and regulation of expression of several lipid-metabolizing genes. The objective of this study was to compare the short-term effects of linoleic acid (LA) and conjugated linoleic acid (CLA) isomers (*cis-9,trans-11* and *trans-10,cis-12*) on acyl CoA oxidase (ACO), 3-hydroxy, 3-methylglutaryl CoA reductase (HMGR) and apolipoprotein A-I (Apo A-I) gene expression in HepG2 and H-4-II-E cells. Steady-state levels of ACO, HMGR and Apo A-I mRNA were examined following 24 h incubation in the absence (control) or presence of 100 μ M each of LA, *cis-9,trans-11* CLA or *trans-10,cis-12* CLA. Concentrations of ACO, HMGR and Apo A-I mRNA transcripts were greater in HepG2 cells treated with *trans-10,cis-12* CLA than in control cells. In the rat hepatoma cell line, ACO gene expression was increased by all three PUFAs. Co-incubation with MK886, a selective inhibitor of peroxisome proliferator-activated receptor alpha (PPAR α), had minimal effects on ACO, HMGR and Apo A-I mRNA responses to CLA in HepG2 cells. Results indicate that the *trans-10,cis-12* CLA isomer is a potent stimulator of ACO, HMGR and Apo A-I gene expression in human and rat hepatocytes. Consistent with the low expression of PPARs in HepG2 cells, selective inhibition of PPAR α had minimal effects on basal or CLA-induced lipid-metabolizing gene expression in the human hepatoma cell line.

EFFECTS OF A BIODEGRADABLE DESLORELIN IMPLANT ON UTERINE INVOLUTION, INFLAMMATORY RESPONSES AND OVARIAN ACTIVITY IN POSTPARTUM LACTATING DAIRY COWS

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The objectives were to evaluate effects of increasing doses of DESL implants on ovarian follicular suppression, uterine involution and degree of inflammation during the days postpartum (dpp). Holstein cows randomly received subcutaneously one (DESL1, n=15) or two (DESL2, n=14) biodegradable DESL implants (2.1 mg) within 0.5 to 1.5 dpp for comparison to control cows (CON, n=18). Enrollment consisted of normal cows (no dystocia, stillborns and milk fever) with BCS=2.75. Cows diagnosed with retained fetal membranes were included. Ultrasound was used to monitor number of ovarian follicles (Class 1, <5 mm; Class 2, 6-9 mm; Class 3, =10 mm), number of CL, diameters of previous pregnant (PH) and non-pregnant (NPH) uterine horns, and diameter of cervix on 8 ± 1, 15 ± 1, 22 ± 1, 29 ± 1, and 36 ± 1 dpp. At 44.5 ± 2.4 dpp, cows entered a pre-synch/Ovsynch protocol: 100 µg, i.m., GnRH (Merial Ltd., Athens, GA) + CIDR (Pharmacia, Kalamazoo, MI) on d0, 25 mg, i.m., PGF_{2a} (Pharmacia Upjohn, Kalamazoo, MI) – CIDR on d7, GnRH on d17, PGF_{2a} on d24, GnRH on d26 and TAI 16h later. Blood samples were collected 3 days/week from 1 dpp to presynch, at PGF_{2a} of pre-synch and at subsequent GnRH (i.e., cycling status), and at TAI + 7d after TAI (i.e., synchrony ovulation) were used for analysis of plasma P₄. Mean number of Class 1 follicles was increased in DESL1 and DESL2 cows (12.1 ± 0.73, 12.9 ± 0.86 > 8.32 ± 0.6; P< 0.01) and decreased frequencies of Class 2 (3% and 2.75% < 34%, P< 0.01) and Class 3 follicles (3% and 2.76% < 47.8%, P< 0.01) as well as CL (0%, 0% < 15%, P < 0.01). DESL1 and DESL2 implants reduced mean diameter of the PH (2.64 ± 0.08, 2.58 ± 0.10 < 2.95 ± 0.09; P<0.01), NPH (2.20 ± 0.05, 2.11 ± 0.05 < 2.42 ± 0.06; P<0.01), and cervix (3.67 ± 0.08, 3.53 ± 0.1 < 3.82 ± 0.08; P< 0.05). A series of orthogonal day contrasts indicated that DESL treated cows completed physical involution of the PH earlier than CON (i.e., 22 < 29 dpp, respectively). Accumulated plasma P₄ (ng/ml) was reduced in the DESL treated groups during the period of 44.5 ± 2.4 dpp (1.33 ± 0.12 < 3.54 ± 0.16; P< 0.01). Number of cows having first ovulation prior to presynch, based upon plasma concentrations of P₄, was reduced in DESL groups (7.14% [2/28] < 61.1% [11/18], P<0.01). First ovulation was delayed in DESL groups (43.5 ± 3.5 > 28.6 ± 7.3 dpp; P<0.01). The DESL treatments reduced the frequency of cows cycling during presynch (14.81% [4/27] < 94.42% [17/18], P<0.01) and that ovulated following Ovsynch (37% [10/27] < 89% [16/18], P<0.01). The bovine uterus is a primary source of F series prostaglandins during the early dpp (Guilbault et al., 1984). Massive release of PGF_{2a} is a result of an inflammatory process in the uterus (Del Vecchio et al., 1992). The 15-keto-13, 14-dihydro-prostaglandin F_{2a} (PGFM) can be used as an index of uterine PGF_{2a}. Acute phase proteins such as haptoglobin are hepatocyte-derived substances induced by cytokines (i.e. interleukin-1 – 6 and TNF- α) upon bacterial endotoxin stimulation. For future accomplishments plasma samples collected every other day for the first 14 dpp will be used to analyze the concentration of PGFM and haptoglobin. A radioimmunoassay procedure will be used and counted in a spectral liquid scintillation for analysis of plasma concentration of PGFM. Haptoglobin will be analyzed by its haemoglobin-binding capacity using the method of 96-well microtitre plates, color absorbance will be counted (Regassa and Noakes, 1999). Use of a biodegradable DESL implant during the postpartum period can enhance uterine involution and possibly reduce degree of inflammation. Although, induction of a prolonged anovulatory period is a negative factor that warrants further investigation (i.e., evaluate dose and duration of DESL implants treatment), the present system provides a potential experimental model to examine gene expression in the rapidly involuted uterus.

EXPRESSION OF SPERM PROTEIN AT 22KDA (SP22) IN EQUINE TISSUES

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SP22 (sperm protein 22KDa) has been correlated with fertility in rats and has also been shown to be present in many non-reproductive tissues in the rat. We have previously identified SP22 on equine spermatozoa and found that the localization patterns of the protein were altered on sperm following cryopreservation and thawing. However, it is not known if SP22 is specific to spermatozoa or if this protein is expressed in other equine tissues as well. The objective of this report was to identify SP22 mRNA and protein in multiple equine tissues. Tissue samples were collected from three adult mares that were being euthanized for other purposes. The tissues collected were heart, skeletal muscle, liver, lung, intestine, kidney, spleen, and fat. All tissues were snap frozen in liquid nitrogen and stored at -80C before used. Tissues underwent extraction using Trizol reagent. The RNA fractions were used in Reverse Transcriptase-PCR (RT-PCR) and the protein fractions were used in Western Blotting. RT-PCR was performed using gene specific primers developed from a homology between known rat and human sequences. Western Blotting was performed using a primary SP22-antibody and FITC-conjugated secondary antibody. The bands were visualized using Chemiluminescent Substrate. The RT-PCR results showed that gene expression was present in all tissue samples. Western Blotting detected protein in heart, skeletal muscle, liver, kidney, and spleen, but not in lung intestine and fat. Although SP22 has been correlated with fertility, the mechanism has not been determined. The wide spread expression of SP22 mRNA in all tissues examined and the secretion of the protein in only a portion of these tissues, raises questions about the function of SP22 in reproduction and other organ systems.

THE USE OF MESENCHYMAL STEM CELLS (MSC) AS A CURE FOR OSTEOPOROSIS

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Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures, especially of the hip, spine and wrist accelerating approximately 10 years following menopause in women and after 70 in men. It is a common human disease affecting millions of people each year. Various treatments are frequently being used today to prevent bone loss but none are effective at treating cases in which bone loss already exists. New therapies for osteoporosis that promote bone rebuilding such as parathyroid hormone (PTH), sodium fluoride, and basic fibroblast growth factor (bFGF) are now being studied in rats. Ovariectomized rats have been shown to be a good model for bone loss in postmenopausal women due to similarities in bone loss due to estrogen deficiency. In both the human and rat, rapid bone loss occurs in the first 5-10 years after estrogen deficiency. Though the treatments being tested in the ovariectomized rats do show some promise in bone rebuilding, they are not effective enough to treat severe cases of osteopenic bone. It is believed that some of the reasons for the ineffectiveness of the bone rebuilding treatments could be due to the decrease of MSCs during ageing. This study will examine whether the addition of MSCs to osteopenic rats results in MSC targeting to osteopenic bone and bone rebuilding. In order to test this hypothesis, we will create two retroviral and two adeno-associated viral (AAV) vectors with fluorescent reporters and transfect them into MSCs. The first will allow detection of cell residence using ubiquitous expression of green fluorescent protein (GFP), while a second fluorescence protein will define differentiation into osteoblasts, the bone forming cells. Additionally, the effectiveness of the two vector types will be evaluated for eventual use in osteoporosis therapies. It is expected from these experiments that the MSCs injected into rats will be able to differentiate *in vivo* and begin to lay down new bone. If this study shows that MSCs introduced into osteopenic rats allow for osteoblasts to be replaced and bone regeneration, the information gained could be used to develop a cure for osteoporosis in human patients. Though many treatments are now being used to prevent bone loss in postmenopausal women, this would be the first treatment that would allow for bone regeneration in cases of osteoporosis in which bone mass is already lost. This study could prove to be beneficial to millions of people throughout the world who suffer from severe osteoporosis. (supported by NIH/NIA R37AG0924)

ACTIONS OF PROGESTERONE ON UTERINE IMMUNOSUPPRESSION AND GLAND DEVELOPMENT IN UTERINE GLAND KNOCKOUT (UGKO) EWE

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Progesterone inhibits immune function and prolongs survival of allografts placed within the uterine lumen. In sheep, one mediator of this effect is believed to be ovine uterine serpin (OvUS), a 55 – 57 kDa protein secreted by endometrial epithelium under the influence of progesterone. Development of glands in the neonate can be inhibited by neonatal exposure to norgestomet, which produces an adult UGKO phenotype. The objective of this experiment was to determine if the UGKO uterus is able to maintain skin grafts in response to progesterone. Control and UGKO ewes (n=8/treatment) were ovariectomized and then treated with 100 mg/day of progesterone or corn oil vehicle for 30 days. An allograft and autograft of skin were then placed in each uterus and 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 present in the uterus for all progesterone-treated control and UGKO ewes. Histological analysis indicated grafts had a necrotic appearance. Thus, progesterone delayed, but did not prevent allograft rejection even in the UGKO ewes. In the absence of progesterone, uterine glands were absent or minimally present in UGKO ewes. As expected uterine glands were present in all control ewes and unexpectedly in 8/8 UGKO ewes treated with progesterone. Thus, long-term progesterone treatment was able to induce differentiation and development of functional endometrial glandular epithelium, presumably from stem cells resident within the endometrial luminal epithelium. Indeed, OvUS was present in the uterine lumen of all progesterone-treated ewes and was localized immunohistologically to both the glandular and luminal epithelium. Results confirm the finding that progesterone delays rejection of allografts within the uterine lumen and demonstrate that this action is not lost in the UGKO phenotype. The finding that progesterone reinitiates glandular development and that such glands are competent to produce progesterone-induced proteins indicates the usefulness of the UGKO model for studying uterine morphogenesis. (Support: USDA NRICGP Grant # 2001-35204-10797 and 2001-02259)

DNA METHYLTRANSFERASE 1 AND 3a GENE EXPRESSION IN BOVINE OVARIAN, TESTES AND SKIN FIBROBLAST CELLS

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To date somatic cell nuclear transfer (NT) embryos experience low survival rates to term. Studies have indicated that epigenetic modifications of the genome may contribute to this low survival rate. Success in somatic cell NT depends in part on the reprogramming of the donor cell nucleus so that it can resume gene expression similar to that of in vivo embryos. DNA methylation is one epigenetic modification that may be a cause for abnormal development of somatic cell NT embryos. Normally, the paternal genome of in-vitro fertilized embryos is rapidly demethylated shortly after fertilization whereas, the maternal genome is more resistant and methylation is gradually lost in a passive or cell cycle dependent manner until the 8-16 cell stage, when both maternal and paternal genomes are remethylated; a pattern not observed in cloned embryos. Cloned embryos exhibit incomplete demethylation during this early stage of development. DNA methylation is catalyzed by enzymes known as DNA methyltransferases (DNMT). DNMT3a is responsible for de novo methylation and DNMT1 is known to be the maintenance methyltransferase. Previous studies have shown that DNMT1 mRNA levels are higher in lower passage cells and decrease over time in culture, whereas the opposite has been shown for DNMT3a; its mRNA expression is low in early cell passages and increases in higher cell passages. The aim of this study was to determine if age, sex and cell type have an affect on DNMT1 and DNMT3a gene expression in cultured cells. Variation in DNMT expression could result in the aberrant methylation seen in cloned embryos. Primary fibroblast cultures were produced from tissue explants from ovary, testes and skin cultured in DMEM supplemented with 15% FBS, L-glutamine, nonessential amino acids, and β -mercaptoethanol at 39°C in a humidified environment of 5% CO₂ in air. Total RNA from passage 4, 8 and 12 of each cell line was harvested, reverse transcribed and cDNA was utilized in Real Time PCR, to quantitate DNMT1 and DNMT3a for each cell line. Ovarian and testes cells displayed the greatest relative expression of DNMT. Testes cells displayed the normal trend of a decrease in DNMT1 message ($p= 0.06$) and an increase in DNMT3a message ($p< 0.1$) from low to high passage. Ovarian cells however, possessed an opposite trend, with an increase in DNMT1 expression ($p< 0.05$) from low to high passage. There were no differences in DNMT1 or DNMT3a expression in male skin cells ($p> 0.1$). Furthermore, there was no significant increase in DNMT1 expression in female skin cells ($p> 0.1$); however, there was an increase in DNMT3a expression with increased cell passage ($P< 0.1$). There were no significant differences between the two sexes ($p> 0.1$) for DNMT1; however for DNMT3a, there was a significant difference, with female cells displaying higher levels of DNMT3a ($p<0.05$). This work demonstrates that DNMT expression is altered during extended culture conditions and is dependent upon cell type. Cell culture can greatly affect gene expression in bovine somatic cells utilized for nuclear transfer, and therefore may explain some of the variability seen in properly reprogramming cloned embryos produced by somatic cell nuclear transfer.

EFFECTS OF BOVINE SOMATOTROPIN (bST), PREGNANCY AND A DIET ENRICHED IN OMEGA-3 FATTY ACIDS ON ENDOMETRIAL GENE EXPRESSION AT DAY 17 AFTER OVULATION IN LACTATING DAIRY COWS

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Objective was to examine the effects of bST injection, pregnancy and dietary fatty acid profile on the expression of various uterine endometrial genes regulating prostaglandin synthesis in lactating dairy cows. Two diets were fed, starting 18 days postpartum (PP), in which the oil of whole cottonseed (15% of dietary DM; control diet; n=19) was compared to oil prepared as a calcium salt containing palm and fish oils (FO), high in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as the primary oils (1.9% of dietary DM; n=8). The calculated total intake of EPA and DHA was 14.8 g/d. All cows started an Ovsynch protocol between d 5 and 12 of an estrous cycle. Cows were assigned randomly to be time inseminated (d 0; approximately 77 d PP) or not, to a bST treatment (500 mg injection at insemination and 11 d later) or not. On d 17 cows were slaughtered, uteri flushed to recover secretions and verify conceptuses, and endometrial tissue collected from the horn ipsilateral to the CL for mRNA and protein analyses. The numbers of animals in each group were as follows: control diet had 5 bST-treated cyclic (C), 5 non bST-treated C, 4 bST-treated pregnant (P), and 5 non bST-treated P cows; FO diet had 4 bST-treated C and 5 non bST-treated C cows. Treatment with bST increased progesterone receptor mRNA levels in C animals fed the control diet but not in FO or P animals (interaction $P < 0.05$). Relative levels of estrogen receptor alpha ($ER\alpha$) mRNA, $ER\alpha$ protein and oxytocin receptor (OTR) mRNA were decreased in P cows compared to C animals, and bST tended to increase OTR and $ER\alpha$ mRNA levels in C cows fed control or FO diets. Steady-state levels of prostaglandin H synthase 2 (PGHS-2) mRNA did not differ among treatments; however, the quantity of PGHS-2 protein was elevated in P cows. Prostaglandin E synthase mRNA levels were elevated in bST-treated C cows fed the control diet and P cows whereas prostaglandin F synthase mRNA concentration decreased in bST-treated P cows. Pregnant animals had more $PGF_{2\alpha}$ in uterine luminal flushings (ULF) than C cows fed the control diet. The bST-treated P cows had more PGE_2 in the ULF than P cows. In summary, both pregnancy and bST altered endometrial gene expression, and cyclic cows responded differently to bST than pregnant cows.

ROLE OF THE INSULIN-LIKE GROWTH FACTORS IN EMBRYO VIABILITY AND SURVIVAL FOLLOWING TRANSFER

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The addition of insulin-like growth factor-1 (IGF-1) to embryo culture medium increases the proportion of oocytes that develop to the blastocyst stage in several species, including cattle (Matsui et al., 1995; Palma et al., 1997). Recently, it was reported that embryos cultured in the presence of IGF-1 are better able to survive following transfer to synchronized recipients (Block et al., 2003). It is not clear whether this represents a general effect of IGF-1 or a heat-stress specific effect since the experiment was conducted in the summer using heat-stressed, lactating cows. Regardless, identification of the mechanisms by which IGF-1 improves the viability of in-vitro produced (IVP) embryos may lead to a better understanding of the molecules important for subsequent survival. We hypothesize that day 7 embryos cultured in the presence of IGF-1 will have an increased expression of genes important for embryo survival. To test this hypothesis, we propose to use suppression subtractive hybridization to identify differentially expressed genes between the two groups of embryos. In addition to IGF-1, IGF-2 may also be important for embryo viability and survival following transfer. As for IGF-1, IGF-2 can interact with the type 1 IGF receptor and IGF-2 is similar in size and structure to IGF-1 (LeRoith and Roberts, 2003). However, the effects of IGF-2 on IVP bovine embryos are less characterized than for IGF-1. We hypothesized that the addition of IGF-1 or IGF-2 to bovine embryo culture would increase the proportion of oocytes that developed to the blastocyst stage on day 8 after insemination and that this increase would be observed for culture under both high and low oxygen concentrations. Embryos were produced in vitro as described previously (Block et al., 2003) and cultured in the presence of 100 ng/mL IGF-1, an equimolar amount of IGF-2 (88 ng/mL) or served as untreated controls. Embryos were cultured until day 8 in either high oxygen (5% CO₂ in humidified air) or low oxygen (5% CO₂, 5% O₂, and 95% N₂). Cleavage rate was recorded on day 3 after insemination and the proportion of oocytes that developed to the blastocyst stage was recorded on day 8. The proportion of oocytes that underwent cleavage was not affected by culture treatment or oxygen concentration. In addition, there was no interaction between culture treatment and oxygen concentration for blastocyst development. Overall, addition of both IGF-1 and IGF-2 increased the proportion of blastocysts ($p < 0.005$) on day 8 compared to controls ($37.8 \pm 2.0\%$ and $33.7 \pm 2.0\%$ vs. $27.6 \pm 2.0\%$, respectively). Furthermore, oxygen concentration during the culture period also affected blastocyst development ($P < 0.0001$) with more blastocysts produced in low oxygen than high oxygen (38.6 ± 1.5 vs. 27.4 ± 1.7 , respectively). Further research is necessary to determine the mechanisms by which IGF-1 increases embryo viability and survival. Results indicate that the effects of IGF-1 and IGF-2 are not dependent on oxygen concentration during culture and that IGF-2 may be another molecule used to increase embryo survival following transfer.

EICOSAPENTAENOIC ACID INHIBITS PROSTAGLANDIN F_{2α} PRODUCTION THROUGH A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-INDEPENDENT MECHANISM IN BOVINE ENDOMETRIAL CELLS

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Polyunsaturated fatty acids (PUFAs) elicit several physiological changes through alteration of the activity and or synthesis of nuclear peroxisome proliferator-activated receptors (PPARs). The objective of this study was to examine the role of PPAR δ in eicosapentaenoic acid (EPA)-mediated inhibition of prostaglandin F_{2 α} (PGF_{2 α}) production in cultured bovine endometrial (BEND) cells. Confluent BEND cells were incubated in the absence (control) or presence of 100 μ M of EPA for 24 h. After incubation, cells were rinsed and then stimulated with phorbol 12,13-dibutyrate (PDBu; 100 ng/ml) for 6 h. Additional sets of culture dishes were treated with a combination of EPA, PDBu and a PPAR δ or α inhibitor to examine the potential role of these nuclear receptors in uterine endometrial PGF_{2 α} production. As expected, exogenous PDBu increased PGF_{2 α} production within 6 h. Pre-incubation of BEND cells with EPA reduced PGF_{2 α} response to PDBu by 75%. Addition of a PPAR δ agonist (L-165041) to the culture medium had no detectable effects on basal or PDBu-stimulated PGF_{2 α} secretion. Interestingly, PGF_{2 α} response to PDBu was completely abolished in cells treated with a combination of EPA and a PPAR δ inhibitor. Conversely, co-incubation with a selective PPAR α inhibitor had no effect on endometrial PGF_{2 α} response to EPA. Results extend the previously documented inhibitory effect of EPA on bovine endometrial PGF_{2 α} production and suggest that supplemental EPA may attenuate uterine PGF_{2 α} production via a PPAR-independent mechanism.

PHYSIOLOGICALLY-RELEVANT HEAT SHOCK DISRUPTS MEIOTIC COMPETENCE IN BOVINE OOCYTES

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Meiotic maturation in mammalian oocytes is a complex process which involves extensive rearrangement of microtubules and actin filaments and chromosomal organization. Cytoskeletal elements are known to be heat sensitive. Therefore, the present study was performed to determine whether physiologically-relevant heat shock during maturation blocks the progression of the oocyte through meiosis and disturbs cytoskeletal reorganization. Cumulus-oocyte complexes (COCs) obtained by slicing follicles were cultured at 38.5, 40 or 41°C for the first 12 h of maturation. Incubation during the last 10 h of maturation was at 38.5°C and 5% (v/v) CO₂ for both treatments. At the end of maturation, oocytes were denuded of cumulus cells by vortexing with hyaluronidase, washed in PBS-PVP, fixed in 4% (w/v) paraformaldehyde. Oocyte were stained with Hoescht 33342 to visualize DNA (Experiment 1) or stained with Hoechst 33342 and markers for either filamentous actin (phalloidin conjugated to Alexa Fluor 594) or microtubules (mouse anti-bovine- α -tubulin labeled with Zenon Alexa Fluor 488; Experiment 2). Labeling was observed using a Zeiss Axioplan fluorescence microscope. For experiment 1, oocytes were classified as being at metaphase I (MI), anaphase I (AI), telophase I (TI) and metaphase II (MII). The percentage of oocytes that were at the MII stage was higher ($P < 0.01$) for oocytes at 38.5°C (n=106) than for oocytes at 40°C (n=110) or 41°C (n=98). In Experiment 2, analysis of microfilaments and microtubules was conducted on oocytes from 38.5°C (n=37) and 41°C (n=50). About 84% percent of the control oocytes were at the MII stage while heat-shocked oocytes were mostly (88%) at MI, AI or TI stages. Oocytes at MII stage were characterized by a small metaphase plate (MP) and the presence of the first polar body. At this stage, microtubules were detected in both the second meiotic spindle and the polar body. A rich domain of microfilaments was formed through the meiotic spindle and around the polar body. In addition, an intense ring of actin was present under the plasma membrane. Microfilaments were also observed to transverse the zona-pellucida. Oocytes at the MI stage were characterized by condensed chromatin, large MP and lack of a polar body. Microtubules within the meiotic spindle were identified in AI and TI stages. At these stages, microfilaments were observed within the oocyte cytoplasm only. The meiotic (MI and MII) spindles in control oocytes were well organized, with sharply defined structures and with chromosomes aligned along their midline. In contrast, spindles of heat-shocked oocytes usually appeared disorganized, especially for those oocytes that did not reach MII. Such aberrant spindles were amorphous, with a more rounded shape, the chromosomes not aligned equatorially, and with microtubules disorganized. In conclusion, heat shock induced disruption in microtubule and microfilament rearrangement associated with arrest at MI and impaired formation of the meiotic spindle. These alterations are likely to be involved in the mechanism underling heat-shock induced disruption in competence of bovine oocytes. Support: BARD FI-330-2002, USDA NRICGP 2002-35203-12664.

EFFECT OF OXYGEN TENSION ON HEAT-INDUCED APOPTOSIS AND DEVELOPMENT OF PRE-IMPLANTATION BOVINE EMBRYOS

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Heat shock effects have been shown to be mediated by oxygen concentrations in many different cell types. A series of experiments were performed to test the hypothesis that oxygen tension affects the magnitude of the deleterious effects of heat shock on in vitro produced bovine embryos. In all experiments, oocyte maturation and fertilization were performed under atmospheric oxygen concentration (20.95% O₂; high oxygen). In experiment 1, embryos were cultured in either high or low (5% O₂) oxygen tensions. Those ≥ 16 cells were collected on day 5 after insemination and were cultured at 38.5 °C for 24 h or 41°C for 9 h and 38.5 °C for 15 h. Total cell number was greater in embryos cultured under low oxygen tension. Heat shock increased percentage of blastomeres that were TUNEL-positive ($P < 0.05$) for embryos under high oxygen tension (9% \pm 1.1% TUNEL positive vs 12.7% \pm 1.8% TUNEL positive) but not for embryos cultured under low oxygen tension (8.7% \pm 1.2% TUNEL positive vs 6.3% \pm 1.0% TUNEL positive). Experiment 2 was carried out to verify if the lack of heat shock induced apoptosis in embryos cultured under low oxygen tension was related to different total cell number at the time of collection. Accordingly, embryos were cultured under low oxygen tension and those ≥ 16 cells were collected on either day 4 or 5 after insemination and then heat shocked as before. Total cell number was greater in embryos harvested on day 5 but there was no effect of heat shock on the percentage of blastomeres positive for TUNEL on either day. In experiment 3, 2-cell embryos were heat shocked for 9 hours at 41°C at either high or low oxygen tension. Heat shock disrupted embryo development in both oxygen tensions. In experiment 4, embryos were cultured under high oxygen tension until day 5 when those ≥ 16 cells were collected, reassigned to either high or low oxygen tension and then cultured at either 38.5 °C for 24 h or 41°C for 9 h and 38.5 °C thereafter. After 24 h, all embryos were cultured under high oxygen tension. Heat shock disrupted embryo development ($P < 0.01$) when embryos were heat shocked under high oxygen tension (55.2% \pm 2.6% blastocyst vs 21.5 \pm 2.6% blastocyst) but not when embryos were heat-shocked under low oxygen tension (55.1% \pm 2.6% blastocyst vs 53.5% \pm 2.6% blastocyst). In conclusion, embryonic sensitivity to heat shock depends upon oxygen concentration in a stage-of-development dependent manner. Embryos ≥ 16 cells are resistant to heat shock unless oxygen content is high. At the 2-cell stage, in contrast, heat shock disrupts development regardless of oxygen tension. These data suggest that the mechanism by which embryos at later stages of development use to overcome harmful effects of heat shock is disrupted in the presence of high oxygen tension. (Support USDA NRICGP 2002-35203-12664).

FATTY ACID REGULATION OF PROSTAGLANDIN F_{2α} PRODUCTION IN BOVINE ENDOMETRIAL CELLS

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Evidence is rapidly accumulating that dietary manipulations of polyunsaturated fatty acids (PUFAs) can have major effects on eicosanoid synthesis in domestic animals. Depending on the amount of particular fatty acids reaching the target tissues, supplemental PUFA can either stimulate or inhibit prostanoid synthesis. The objective of this study was to examine the effects of fatty acids with varying degree of unsaturation on phorbol 12, 13-dibutyrate (PDBu)-induced prostaglandin (PG) production in bovine endometrial (BEND) cells. Confluent BEND cells were incubated in the absence (control) or presence of 100 μM of stearic (ST), linoleic (LA), linolenic (LNA) or eicosapentaenoic (EPA) acids for 24 h. After incubation, cells were rinsed and then stimulated with PDBu (100 ng/ml) for 6 h. As previously documented, exogenous PDBu increased PGF_{2α} concentration in BEND cell-conditioned media within 6 h. The increase in PGF_{2α} concentration in the culture medium coincided with significant induction of cyclooxygenase-2 (COX-2) and prostaglandin E synthase (PGES) gene expression in PDBu-treated cells. Priming of BEND cells with EPA decreased PGF_{2α} response to PDBu, but had no detectable effects on COX-2 or PGES mRNA concentrations in BEND cells. Short-term treatment of BEND cells with ST, LA or LNA had no detectable effects on PGF_{2α}, COX-2 or PGES responses to PDBu. Results indicate that supplemental fatty acids may regulate PGF_{2α} production through cellular and/or molecular mechanisms that do not involve COX-2 gene repression.

Timing of In Vitro Oocyte Maturation in Springbok (*Antidorcas marsupialis*), Black Wildebeest (*Connechaetes gnu*), Blesbok (*Damaliscus dorcus phillipsi*), and Reedbuck (*Redunca arundinum*)

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With an increased need for genetic diversity within populations, assisted reproductive technology is becoming an important tool for banking semen and embryos, with the possibility of future AI or embryo transfer between distant populations. The purpose of this study was to determine the timing of maturation in several antelope species. Previous research has demonstrated that the broad approach of applying bovine IVM/F/C protocols to African antelope is inefficient at producing embryos. Antelope analyzed in this study were springbok (*Antidorcas marsupialis*; n=84), black wildebeest (*Connechaetes gnu*; n=18), blesbok (*Damaliscus dorcus phillipsi*; n=9), and reedbuck (*Redunca arundinum*; n=1). Biological materials were collected from culled animals on game reserves in South Africa within 4 h of death. Ovaries were placed into warm SOF-HEPES, sliced and cumulus oocyte complexes (COCs) collected. Complexes were selected on the basis of an even cytoplasm and at least 2 layers of cumulus cells. Selected COCs were placed into GMat supplemented with 0.01 U/ml FSH and LH, 50 ng/ml EGF, and 1.0% (v/v) PSA. Oocytes were removed from medium after 16, 20, 24, 28, or 32 hours of maturation in 5% CO₂ in air at 39°C. After removal, complexes were denuded in hyaluronidase, mounted on a slide and placed into 3:1 (ethanol:glacial acetic acid) fixative until time of analysis. Oocytes were stained with aceto-orcein, and nuclear maturation was evaluated on a phase contrast microscope. Oocytes exhibiting telophase or metaphase II were classified as mature. In springbok (n=311), there was no difference (P > 0.05) in maturation to MII after 28 h. Fewer springbok oocytes were mature at 16, 20, and 24h. Black Wildebeest (n=88) oocytes reached MII by 24h, with no additional increase in maturation at 28 or 32h. Blesbok oocytes (n=42) were not mature at 16 or 20h, and there was no difference (P > 0.05) in the percent of oocytes mature at 24 or 28h. Observational data in reedbuck (n=10) indicated that no oocytes were mature at 20 or 24h, but 25.0% were mature at 28h. Low occurrence of MII at 28h in blesbok and reedbuck suggests that additional later timepoints should be examined. This study demonstrates that oocytes of these species can be successfully matured in vitro, but the rate of maturation and thus the optimal time of insemination for IVF is species specific.

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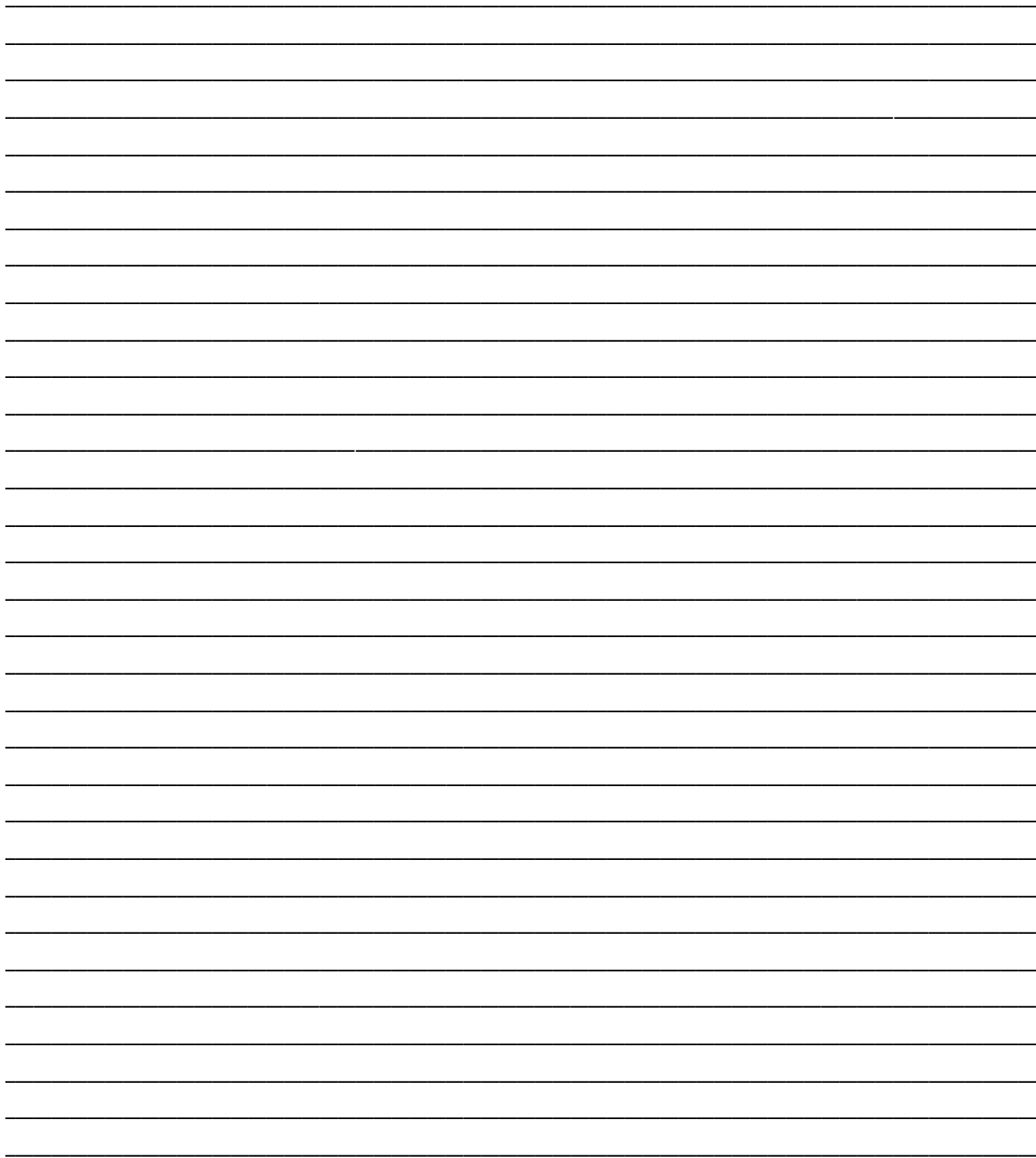
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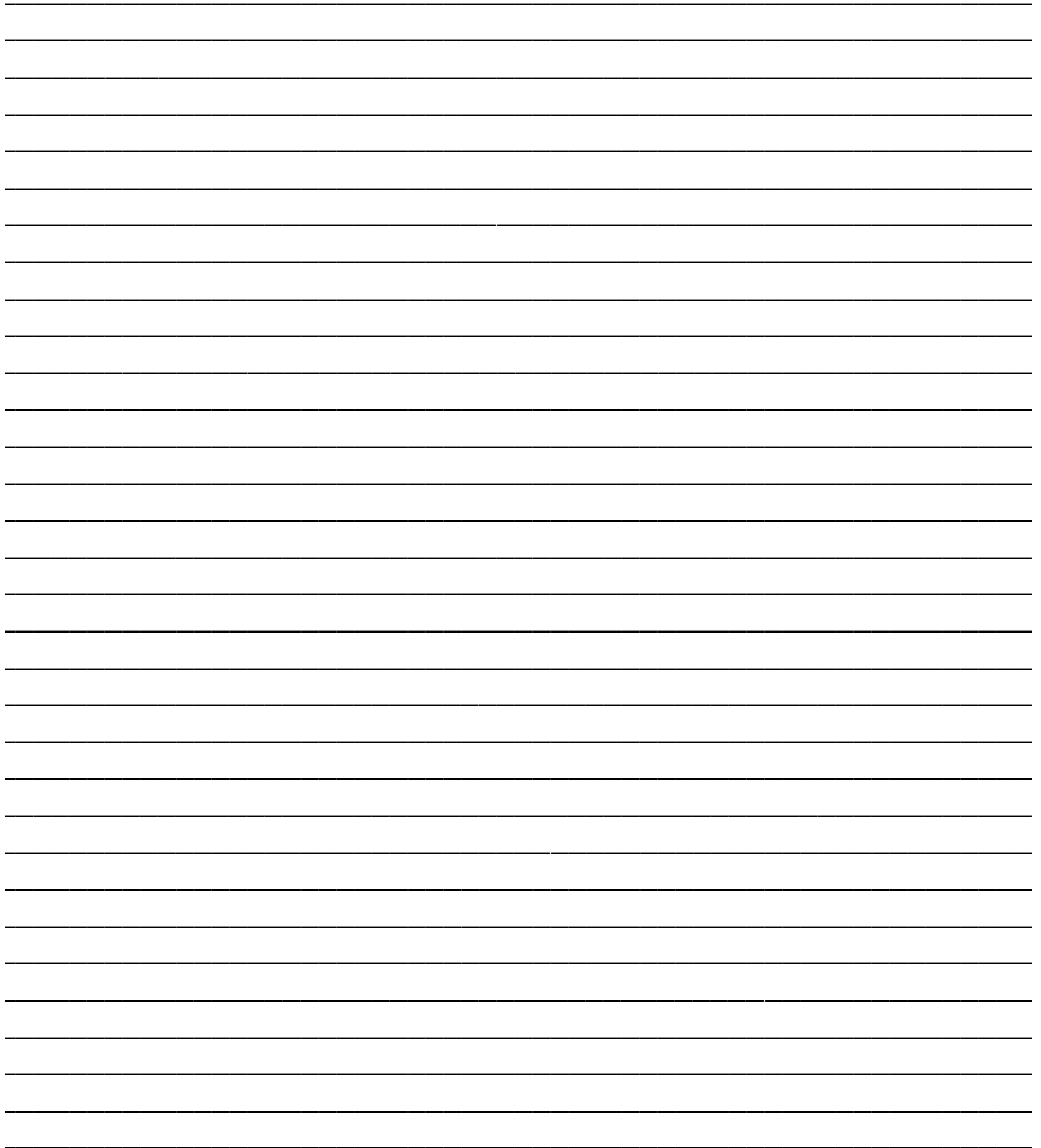
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