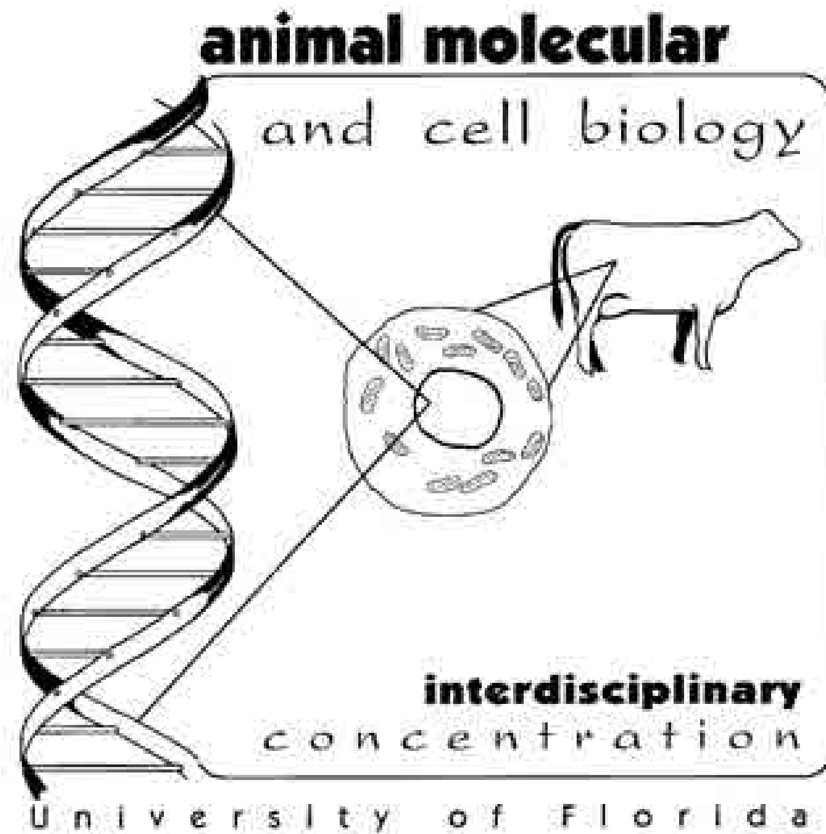
University of Florida

Animal Molecular and Cellular Biology

Fourth Annual Research Symposium



Lake Wauberg, Gainesville, Florida

April 7th and 8th, 2006

Animal Molecular and Cellular Biology
Fourth Annual Research Symposium

WELCOME

It is our great pleasure to welcome you to our Fourth Annual Research Symposium for the Animal Molecular and Cellular Biology Interdisciplinary Graduate Program, University of Florida, held at Lake Wauberg. This two-day event has been planned 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. David Julian, and our Distinguished AMCB Lecturer, Dr. Ina Dobrinski, from the University of Pennsylvania. 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.

Karen Moore, AMCB Director
Bill Buhi, AMCB Co-director

ACKNOWLEDGEMENTS

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

Dr. Mark McLellan, Dean of Research, IFAS

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

Dr. Jimmy Cheek, Vice President, IFAS

Lake Wauberg

Lake Wauburg North and South are outdoor recreation park facilities owned and operated by the University of Florida. The lake sides offer quiet places to relax, and have fun. The North Park is located eight miles from campus on Highway 441 near Payne's Prairie. Wauburg North is beautifully landscaped with 25 acres of shaded, grassy picnic areas, complete with picnic tables and grills on a hillside setting overlooking the lake and swimming area. Canoes, kayaks, paddle boats and rowboats are available and can be checked out for free with your Gator-1 Card. Beach volleyball and fishing from the dock are also popular activities.

History of Lake Wauberg:
In 1918, the University YMCA purchased twenty acres of land and water at Lake Wauburg as a recreation center for University of Florida students. This was before the road across Payne's Prairie was completed. The journey to the lake took about a half-day on a winding road of mostly sand with the danger of getting stuck in the sand and mud. In 1928 Dr. John Tigert became president of the university. The University YMCA dissolved, and the land was deeded to the University of Florida with the understanding that the Union would administer the facility. The Union took over the running of Lake Wauburg (then Camp Wauburg) in 1936, and the transfer of the property to the University of Florida occurred in 1938. In 1939 a recreation center and a small residence were built on the property. Today both are renovated and currently in use. The university administration never agreed to put money into Lake Wauburg, so the Union picked up all costs for the administration of the facility. The Union sought support from the university administration and a 35-cent per capita fee for operational costs, both were denied. The Union closed Lake Wauburg, to the public in the fall of 1970 due to a lack of funds and the deterioration of the facilities. Lake Wauburg North reopened in 1974, under the control of the Department of Intramural Athletics and Recreation. The reopening was funded by a reserve fee allocation, which was matched by other funds from within and outside the university. President York agreed to provide normal physical plant services for maintenance. The North Park has been continually operated since 1974 with Student Government funding and assistance from the College of Health and Human Performance. Extensive renovations took place from 1998-2000. A large rest room with showers and changing areas was built in the North Park centrally located to the swimming area and open pavilion. A shelter for boats, equipped with a lifeguard observation tower, was built on the waterfront. The most popular improvement was the renovating of Cypress Lodge. Central heat and air-conditioning and a full service kitchen were added and the recreation room is about 30 percent larger and the room was paneled with pecky cypress. In order to support these new facilities a new water and septic system was installed. The University of Florida's Lake Wauburg Recreation Center is staffed with trained, responsible students and is equipped with the latest in safety and general park materials. The park is known as one of the University's best-kept secrets and will continue to improve with the help of the student body.

SYMPOSIUM AGENDA

Friday, April 7, 2006

9:30AM – Ina Dobrinski, Distinguished Lecturer, *Testis tissue transplantation - A Comparative Approach to the Study of Mammalian Spermatogenesis*
10:30 AM – Transport to Lake Wauberg Cypress Lodge
11:00 AM – Welcome and Opening Remarks
11:10 AM – Student Presentations Session I – Moderator: Ewa Wroclawska
    11:10-11:30 Luiz Augusto de Castro e Paula
    11:30-11:50 Kathleen Pennington
    11:50-12:10 Lilian Oliveira
12:10-12:20- AMCB Group Photo
12:20 PM – Lunch/Break at the Lake (Hosted by AMCB Faculty)
1:20 PM – Dr. David Julian, Invited Faculty Speaker, *The physiology and ecology of oxygen toxicity*
2:15 PM - Student Presentations Session II – Moderator: Sarah Reed
    2:15-2:35 Amber Brad
    2:35-2:55 Michelle Eroh
    2:55-3:15 Andria Desvousges

3:15 PM – Break/AMCB Faculty Meeting

3:45 PM – Student Presentations Session III– Moderator: Dean Jousan
    3:45-4:05 Barbara Loureiro
    4:05-4:25 Aline Bonilla
    4:25-4:45 Dean Jousan

4:45 PM – Volleyball/Canoeing at the Lake
6:00 PM-Midnight - Barbecue and Social at Joe's Deli (13th Street Location; Hosted by AMCB Faculty)

Saturday, April 8, 2006

8:00AM – Breakfast (Hosted by AMCB Faculty) Bldg 459 102
8:30 AM - Student Presentations Session IV– Moderator: Luiz Augusto de Castro e Paula
    8:30-8:50 Maria Padua
    8:50-9:10 Teresa Rodina
    8:50-9:10 Jeremy Block
9:10-9:25 Break
    9:25-9:45 Flavio Silvestre
    9:45-10:05 Katherine Hendricks
    10:05-10:25 Justin Hayna

10:25 AM - Wrap Up
11:00 AM – Adjourn

INVITED SPEAKERS

2006 Distinguished AMCB Lecturer, Dr. Ina Dobrinski

Testis tissue transplantation - A Comparative Approach to the Study of Mammalian Spermatogenesis

Ina Dobrinski holds a veterinary degree from the School of Veterinary Medicine in Hannover, Germany, an MVSc degree from the University of Saskatchewan, Canada, a PhD in Reproduction from Cornell University, NY, and is board certified by the American College of Theriogenologists. She currently is an Associate Professor of Large Animal Reproduction, holds the Marion Dilly and David George Jones Chair in Animal Reproduction, and is the Director of the Center for Animal Transgenesis and Germ Cell Research at the University of Pennsylvania's School of Veterinary Medicine. Research in her laboratory is focused on male germ cell biology in domestic animals and non-human primates. One aspect of this work is the exploration of germ line stem cell biology to develop a new approach to transgenesis in domestic animals through the manipulation of the male germ line.

Contact information:

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2006 AMCB Invited Faculty Speaker, Dr. David Julian

The physiology and ecology of oxygen toxicity

David Julian is a comparative, ecological physiologist with an interest in the cellular responses and adaptations of animals to extreme environmental stressors. To an ecological physiologist, a stressor is any environmental condition that threatens an organism's survival by pushing it outside its normal homeostatic boundaries; that is, any condition that limits an organism's ability to regulate its physiological processes. The core focus of my lab is on the adaptations that allow "extremophiles" to thrive in seemingly inhospitable environments, with a specific emphasis on the cellular and molecular adaptations that allow them to avoid catastrophic damage that would otherwise lead to widespread cell death. We use a variety of animal and cell models in my lab, focusing primarily on marine worms and clams tolerant of hydrogen sulfide and/or hypoxia.

He received a BS and MS in Physiology at San Francisco State University, where he studied the adaptations of marine worms to hydrogen sulfide with Alissa Arp, after which he went to UCSF for a PhD in Physiology with Juan Korenbrot, where he studied the developmental biology of stem cells in the vertebrate (fish) retina. While at UCSF, he spent his "vacations" participating in a number of deep-sea expeditions to hydrothermal vents and hydrocarbon seeps with Charles Fisher at PSU. After a post-doc in invertebrate biochemistry in Duesseldorf, Germany working on marine worms and the nematode *C. elegans* with Manfred Grieshaber, he was hired by the Dept of Zoology at UF, where he has a lab filled with bright, energetic students.

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ABSTRACTS

CERAMIDE INDUCES APOPTOSIS AND DECREASES DEVELOPMENT OF CULTURED BOVINE EMBRYOS

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Ceramide is a second messenger that is induced by stress in different cell types and is known to play a major regulatory role in apoptosis. The objective of the present study was to test the hypothesis that an exogenously administered, cell-permeable synthetic ceramide analogue, C(2)-ceramide, induces apoptosis and impairs development of bovine embryos. Embryos produced in vitro were cultured in KSOM-BE2 under 5% CO_2 in air at 38.5ºC until Day 5 after insemination when embryos >16 cells were harvested and randomly assigned to one of five treatments: 50 µM C(2)-ceramide for 9 hours followed by 15 hours in ethanol vehicle (C2-9), 50 µM C(2)-ceramide for 24 hours (C2-24), 50 µM of an inactive C(2)-ceramide analogue, C(2)-dihydroceramide, for 9 hours followed by 15 hours in ethanol vehicle (DC2-9), or 50 µM C(2)-dihydroceramide for 24 hours (DC2-24), or 24 hours in ethanol vehicle (0.34% v/v; control). In Experiment 1, embryos were fixed 24 hours after beginning of treatment and percent apoptosis was determined using TUNEL assay. Total cell number was decreased in C2-24 group (42±4.2) when compared to control (59.4±4.6), DC2-9 (65.9±4.6) and DC-24 (62.1±4.2) groups ($p<0.05$), but did not differ from C2-9 group (51.6±4.7; $p>0.05$). Apoptosis was increased in the C2-24 group (36%±4.3) when compared to other groups (control, 8.6%±4.7; DC2-9, 9%±4.7; DC2-24, 8.9%±4.2; C2-9, 15.6%±4.8) ($p<0.05$). In experiment 2, embryos were washed 24 hours after beginning of treatment and placed in fresh KSOM-BE2. Percent blastocyst was determined as the percent of embryos harvested on Day 5 becoming blastocysts on Day 8. Percent blastocyst was decreased in C2-24 group (9.54%±4.7) when compared to the other groups (control, 73%±4.7; DC2-9, 74.2%±4.7; DC2-24, 71.7%±4.7; C2-9, 77%±4.7) ($p<0.001$). In conclusion, treatment of bovine embryos with ceramide caused an increase in the percent of TUNEL positive cells and decreased embryo development to the blastocyst stage. These findings raise the possibility that ceramide could be involved in embryonic death caused by stresses like heat shock and ultraviolet radiation that increase ceramide synthesis.

FORMATION OF BINUCLEATE CELLS IN BOVINE TROPHECTODERM

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Department of Animal Sciences, University of Florida, Gainesville Florida

The peri- and post-attachment bovine placenta contains two distinct trophoblast cell types, termed mononucleate and binucleate cells. The binucleate cells (BNC) appear after attachment. These cells comprise approximately 20% of the trophoblast throughout pregnancy and their numbers decline shortly before calving. BNC develop from mononucleate cells throughout gestation by acytokinetic mitosis. In the bovine, BNC migrate and form trinucleated feto-maternal hybrid cells with the uterine epithelium, which eventually lyse, creating the feto-maternal syncytium. The syncytium is thought to provide metabolites for the early developing conceptus. In addition, BNC function to produce and deliver hormones, including placental lactogen and pregnancy-associated glycoproteins, to the maternal circulation. Bovine BNC are morphologically and functionally similar to murine trophoblast giant cells and human extravillous cytotrophoblast, which also invade the uterine lining and deliver various placental-derived hormones to the maternal circulation. The basic helix-loop- helix (bHLH) transcription factor, heart and neural crest derivative 1 (Hand1), has been implicated in controlling trophoblast giant cell and binucleate cell differentiation in some mammalian species. In the mouse, Hand1 loss-of-function prevents giant cell differentiation. Further, overexpression of Hand1 in cultured murine trophectoderm promotes giant cell formation. Hand1 expression has been localized to BNC in bovine placenta throughout pregnancy. This laboratory has isolated Hand1 cDNA from the bovine placenta using polymerase chain reaction (PCR). It is possible that Hand1 and potentially other bHLH molecules serve to regulate BNC formation in cattle and other ruminants. The goal of this research is to identify schemes to induce the formation of bovine binucleate cells in culture. The first approach is to determine if overexpressing bovine Hand1 in cultured CT-1 cells, derived from bovine trophectoderm, will induce BNC formation. Currently, the Hand1 cDNA is being cloned into a mammalian expression vector designed to create a c-myc-tagged fusion protein. This expression vector also contains a strong CMV promoter, which should provide a strong transgene expression. Immunohistochemistry will be used to determine if Hand1 expression co-localizes with placental lactogen-expressing BNC in culture. The second approach for creating BNC in culture will be to evaluate BNC development under several different environmental conditions, including hypoxia, varying serum composition, and supplementation with putative transforming factors. In summary, inducing formation of BNC in culture will provide insight into how these important placental cells are created during pregnancy as well as provide a tool for investigating the regulation of various placental-specific genes, especially hormones, during pregnancy.

EXPRESSION OF FOXP3 IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) IN COWS

Lilian Oliveira* and P.J. Hansen

Department of Animal Sciences, University of Florida

Pregnancy can be used a model of natural immune tolerance since the fetus expresses paternal antigens that can be recognized by the maternal immune system. Regulatory T cells (T_{reg}) are a subset of T lymphocytes having the phenotype $CD4^{+}CD25\ ^{+}FoxP3^{+}$ and which function to inhibit T cell activation. FoxP3 is a member of the *forkhead* family of transcription factors; mutation of FoxP3 is associated with immunoproliferative disorders called IPEX (X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome) in humans and scurfy in mice (Nat Genet. 2001;27:68-73; Nat Genet. 2001;27:18-20.). It is our hypothesis that T_{reg} cells increase during pregnancy and act to inhibit maternal immune responses against the conceptus. This hypothesis is based on observations from other species. In women, the frequency of $CD4^{+}CD25^{+}$ T cells in the circulation was higher during the first trimester of pregnancy when compared to nonpregnant controls (.J. et al. Clin. Exp. Immunol. 2004 136:373-378.). In addition, the numbers of $CD4^{+}CD25^{bright}$ T cells in decidua were higher for women with spontaneous abortion compared to tissues from women with surgically-induced abortions. The presence of T_{reg} cells in cattle has not been established and it is not known whether number or activity of these cells is regulated during pregnancy. As an initial step to testing the role of T_{reg} cells during pregnancy in cattle, experiments are underway to characterize T_{reg} cells in cattle. A polyclonal antibody generated against human FoxP3 in rabbit is being used to identify the phenotype and functional properties of $FoxP3^{+}$ cells in peripheral blood. Initial studies have shown that FoxP3+ cells exist in the PBMC fraction of blood. This was determined using flow cytometry of saponin-permeabilized PBMC with anti-FoxP3 labeled with FITC-conjugated anti-rabbit $F_{(ab)}2$. PBMC were gated by forward and side scatter (Fig. 1). A total of three cows have been examined to date. The proportion of gated PBMC for individual cows at 64 days post partum, 96 days post partum and 296 days of gestation were 4.8, 4.8, and 8.5% respectively. These data indicate that the antibody against human FoxP3 cross reacts with a molecule expressed on bovine lymphocytes. Subsequent studies will examine the phenotype of $FoxP3^{+}$ cells, their functional properties with respect to immunosuppression, and pregnancy-associated changes in $Foxp3^{+}$ cells in peripheral blood and endometrium.

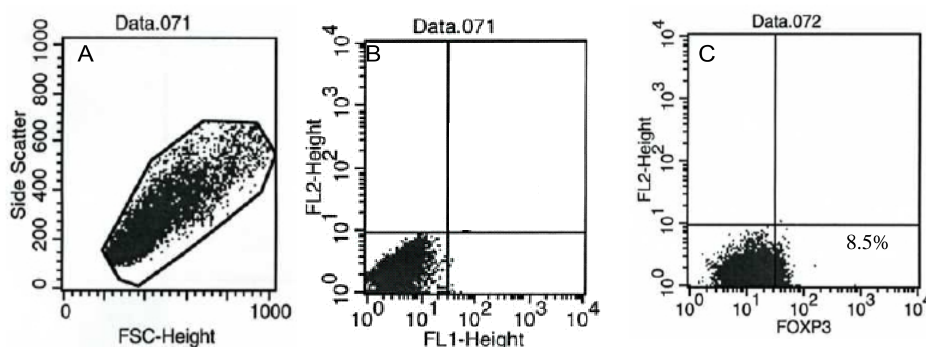

Figure 1: A- Side and forward scatter of bovine PBMC; B- Negative control in FL1 channel for bovine PBMC (only cells); C- Bovine PBMC labeled with FoxP3 polyclonal antibody. Note: the events in lower right quadrant represent positive cells for FoxP3

THE BLOCK TO APOPTOSIS IN BOVINE TWO-CELL EMBRYOS INVOLVES INHIBITION OF EVENTS LEADING TO CASPASE 9 ACTIVATION AND TO GROUP II CASPASE-MEDIATED DNA DAMAGE

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The capacity of the preimplantation embryo to undergo apoptosis in response to external stimuli is developmentally regulated. In the bovine, for example, the two-cell embryo is refractory to induction of apoptosis in response to heat shock, tumor necrosis factor-α, and arsenic. Acquisition of capacity for apoptosis is regained around the 16-cell stage. The purpose of the present experiments was to determine the mechanism by which apoptosis is blocked in the two-cell embryo. In experiment 1, two-cell embryos and embryos > 16 cells at Day 5 after insemination were cultured at 38.5°C or 41°C for 15 h and analyzed for caspase 9 activity using a fluorogenic substrate. Heat shock did not increase caspase 9 activity in two-cell embryos but did increase ($P<0.04$) caspase 9 activity in Day 5 embryos. In experiment 2, embryos were exposed to either 38.5°C or 41°C in the presence or absence of carbonyl cyanide 3-chlorophenylhydrazone (CCCP) to depolarize mitochondria; apoptosis was determined by TUNEL assay at 15 h after initiation of treatment. For two-cell embryos, the proportion of TUNEL-positive blastomeres was not increased by either heat shock (8.6% vs 12.4% TUNEL-positive) or CCCP (0% vs 4% TUNEL-positive). For Day 5 embryos, in contrast, the proportion of TUNEL positive nuclei were increased by CCCP ($P=0.009$; 22.4% vs 49% TUNEL-positive) and heat shock ($P=0.004$; 16.6% vs 26% TUNEL-positive). Experiment 3 was performed like Experiment 2 except that caspase 9 and group II caspases (caspase 2, 3 and 7) were measured using fluorogenic substrates. In two-cell embryos, heat shock did not increase caspase 9 or group II caspase activity, but CCCP increased activity of both caspases ($P<0.001$). In Day 5 embryos, heat shock and CCCP treatment increased group II caspases (caspase 9 not determined). In conclusion, failure of heat shock to increase caspase 9 and group II caspase activity indicates that the signaling pathway leading to mitochondrial depolarization and caspase activation is inhibited in two-cell embryos. The fact that CCCP induced caspase 9 and group II caspase activity indicates that caspase activation is possible following mitochondrial depolarization. However, since CCCP did not increase TUNEL labeling of two-cell embryos, effector actions of caspase 3 and other group II caspases is inhibited.

DEVELOPING A MODEL TO IDENTIFY THE CONCEPTUS-DERIVED FACTOR RESPONSIBLE FOR THE DECLINE IN COX2 EXPRESSION AND PGF2α PRODUCTION DURING EARLY PREGNANCY IN THE MARE.

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The equine embryo must signal its presence from within the uterus to induce a series of events in order for pregnancy to continue to term. This concept is known as the maternal recognition of pregnancy, and remains unresolved in horses. Currently, it is understood that the locomotion of the conceptus throughout the uterus is crucial for its survival during early pregnancy. This movement presumably permits the embryo to transmit its antiluteolytic signal to the endometrium, thereby preventing the production of prostaglandins by the endometrium. Synthesis of the luteolytic prostaglandin F2α (PGF2α) is controlled primarily by cyclooxygenase-2 (COX-2) in the endometrium. Relative expression of COX-2 and production of PGF2α reaches a maximum concentration within the uterus 14 days after ovulation in non-pregnant mares. At this time, PGF2α exerts its luteolytic effect and induces corpus luteum regression, thereby allowing the mare enter a new follicular phase and a subsequent ovulation. During pregnancy, endometrial production of PGF2α is greatly diminished, and the continuing function of the corpus luteum promotes the survival of a conceptus and prevents return to estrus. We propose that the equine conceptus inhibits the production of PGF2α by inhibiting the expression of COX-2 in the equine endometrium. To investigate the role of the conceptus on prostaglandin production, our first aim is to examine the differences in equine COX-2 mRNA abundance in endometrium derived from cycling verses pregnant mares. To accomplish this, RNA will be extracted from endometrial tissues and reverse transcribed. Quantitative real-time PCR will be completed on the reverse transcription product to quantify the relative quantities of COX-2 in samples. Equine COX-2 and 18s cDNAs have been cloned and will serve as positive controls for real-time PCR. Preliminary evidence indicates that COX-2 mRNA expression is diminished in endometrium derived from pregnant mares when compared to those collected from non-pregnant mares. These preliminary findings are being replicated to verify that COX-2 mRNA expression is reduced in endometrium from pregnant mares. A subsequent study will be conducted to determine if co-culturing day 14 non-pregnant endometrium with conceptus membranes diminishes COX-2 mRNA expression. Additionally, cultures of these same endometrial tissues with conceptus-conditioned medium will completed to determine if a soluble conceptus component accounts for the inhibition of COX 2 expression within the uterus. We hypothesize that the in vitro co-culture systems will demonstrate that the presence of a conceptus will elicit a similar reduction in COX2 mRNA expression, inhibiting PGF2α production that is seen in early pregnancy, and can then be used as a model to identify the conceptus-derived factor responsible for the inhibition of COX-2 in the endometrium.

A SELECTIVE ROLE OF SEMINAL PLASMA IN PMN-BINDING AND PHAGOCYTOSIS OF LIVE AND NON-VIABLE EQUINE SPERMATOZOA

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Breeding-induced endometritis is characterized by an influx of polymorphonuclear neutrophils (PMNs) into the uterine lumen. Excess spermatozoa bind to PMNs and become phagocytosed. This is believed to be an important part of sperm elimination from the female reproductive tract in order to provide a compatible uterine environment for the embryo. Seminal plasma has been shown to suppress PMN-binding and phagocytosis of spermatozoa, but it is not known if these effects are selective for live sperm only. We hypothesized that seminal plasma protects viable, but not dead sperm from PMN-binding and phagocytosis. Three stallions were collected three times each, and the spermatozoa were washed and reconstituted in a commercial semen extender (EquiPro, Minitube of America, Verona, WI). Aliquots were stored in room temperature for 1 hr (live), or snap frozen at -80^0 C for 1 hr followed by thawing at room temperature (non-viable sperm). The aliquots were divided into four equal fractions, and one of the following treatments were added to each fraction: 1) seminal plasma 2) semen extender, 3) ammonium sulfate precipitated seminal plasma proteins with protease inhibitor (SPP^+), or 4) ammonium sulfate precipitated seminal plasma proteins without protease inhibitor (SPP^-). PMNs were isolated from peripheral blood from a healthy mare, and samples were subjected to *in vitro* assays for PMN binding and phagocytosis respectively. Results from PMN binding assays were expressed as the percentage of PMNs (mean ± SEM) with at least one spermatozoon bound. Results from phagocytosis assays were expressed as the percentage of PMNs (mean ± SEM) that had phagocytosed at least one spermatozoon. Data was analyzed for normal distribution, and differences between treatment groups were determined by ANOVA and Bonferronis comparison tests. All significance was set at $P<0.05$. Seminal plasma (40±3.1) and SPP^+ (42±3.1) suppressed PMN-binding of live sperm compared to semen extender alone (73±3.1; $P<0.0001$). Similarly, seminal plasma (35±3.0) and SPP^+ (35±3.0) suppressed phagocytosis of live sperm compared to semen extender alone (73±3.0; $P<0.0001$). This effect was also observed, but to a lesser degree in SPP^- treated samples ($P<0.05$). Binding to PMNs was lower for non-viable spermatozoa (38±3.1) compared to live spermatozoa (73±3.1) in the absence of seminal plasma. Phagocytosis of non-viable (24±3.0) spermatozoa was also lower than live sperm (73±3.0) in the absence of seminal plasma ($P<0.0001$). The addition of seminal plasma increased PMN-binding and phagocytosis (71±3.1 and 58±3.0 respectively) of non-viable spermatozoa ($P<0.05$). The addition of SPP^- to samples also increased PMN-binding and phagocytosis of non-viable spermatozoa (50±3.1 and 46±3.0 respectively; $P<0.05$), but to a lesser degree than for seminal plasma treated samples ($P<0.05$). The addition of protease inhibitors removed this effect from SPP. This study demonstrates for the first time that seminal plasma selectively protects live sperm from PMN-binding and phagocytosis, and promotes PMN-binding and phagocytosis of non-viable spermatozoa. The results suggest that more than one protein is involved in these functions.

ROLE OF CASPASE-9 AND STAGE OF DEVELOPMENT IN INDUCTION OF APOPTOSIS BY HEAT SHOCK AND TUMOR NECROSIS FACTOR-α IN BOVINE PREIMPLANTATION EMBRYOS

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We tested the hypothesis that heat shock and tumor necrosis factor-α (TNF-α) induce apoptosis in bovine embryos through a caspase-9 dependent mechanism. Experiment 1 determined whether the caspase-9 inhibitor, z-LEHD-fmk, blocks the apoptotic effects of heat shock and TNF-α. Embryos were collected on day 4, 5, and 6 after in vitro insemination and cultured for 24 h in the presence of either 100 µM z-LEHD-fmk reconstituted in 0.5% (v/v) DMSO or vehicle (DMSO) at either 1) 38.5°C for 24 h (control), 2) 41°C for 15 h followed by 38.5°C for 9 h (heat shock), or 3) 38.5°C for 24 h with 10 ng/mL murine TNF-α. The proportion of blastomeres undergoing apoptosis was determined using TUNEL labeling. The experiment was replicated 4-5 times/day using 172-248 embryos/day. In control embryos, heat shock and TNF-α increased the proportion of cells that were TUNEL-positive as compared to embryos cultured at 38.5°C for 24 h ($P<0.0001$). For embryos incubated with z-LEHD-fmk, in contrast, neither heat shock nor TNF-α caused an increase in TUNEL labeling (treatment x inhibitor; $P= 0.01$). Experiment 2 tested effects of different concentrations of z-LEHD-fmk on apoptotic responses of embryos to TNF-α. Embryos ≥16 cells were collected on Day 6 and cultured in the presence of different concentrations of z-LEHD-fmk (0, 1 10, and 100 µM) ± 10 ng/mL TNF-α.. After 24 h at 38.5°C, embryos were washed, fixed, and analyzed by TUNEL. The experiment was replicated 4 times (176 embryos). Addition of 10 ng/mL TNF-α increased the percentage of cells that were TUNEL-positive and this increase was blocked by all concentrations of z-LEHD-fmk (concentration x inhibitor; $P<0.05$). For Exp. 3, we analyzed whether heat shock increases caspase-9 activity and whether z-LEHD-fmk blocks this increase. Embryos at the morula or early blastocyst stage were collected on day 6 and transferred to a new drop with 100 µM z-LEHD-fmk or DMSO vehicle. Embryos were then cultured at either 38.5°C for 24 h or 41°C for 15 h followed by 38.5°C for 9 h. At the end of heat shock, embryos were washed and then incubated in a caspase-9 substrate (5 µM CaspaLux 9-M_1D_2) at room temperature for 1 h. Caspase-9 activity was then determined by evaluation of fluorescent intensity. Embryos were classified as having low, medium or high caspase activity. The experiment was replicated 5 times using 22-26 embryos/treatment. Heat shock increased the percent of embryos classified as having medium and high caspase-9 activity and z-LEHD-fmk blocked this effect (treatment $P<0.05$; inhibitor $P<0.001$). For Exp. 4, effects of TNF-α on caspase-9 activity were evaluated. Embryos at day 6 were cultured at 38.5°C for 3, 6, 9, 12, and 15 h with 10 ng/mL TNF-α or vehicle and then caspase-9 activity was measured as described before. The experiment was replicated 8 times using 37-39 embryos/treatment. The proportion of embryos classified as having medium or high caspase activity was increased by TNF-α at all time points except 3 h (treatment $P< 0.05$). In conclusion, activation of caspase-9 dependent pathways is involved in the induction of apoptosis by heat shock and TNF-α.
(Supported by USDA Grant No. 2004-34135-14715 and BARD Grant No. US-3551-04).

THE EFFECT OF DIETARY SUPPLEMENTATION WITH PROTEINATED SALT ON SEVERAL REPRODUCTIVE PARAMETERS IN NELORE BULLS

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Several factors can affect bovine male reproduction, nutrition being one that plays an important role. Diets based exclusively on pasture grazing are subject to alterations in the amount and quality of forages available. In tropical conditions this variation and nutritional deficiency happens mainly during the dry season, when the pastures become more fibrous with low digestibility, and little energy, protein and nutritional value. Previous research has shown that for animals under grazing conditions, nutritional supplementation can be used to improve reproductive capacity, including prepubertal testicular growth in order to assure appropriate testicular size at maturity. Moreover, supplementation of adult animals on low quality pastures can stop the decline in testicular size and reduced sperm production by avoiding or decreasing body weight loss. In the present study proteinated salt was used to verify this effect of supplementation on several reproductive parameters in Nelore bulls under grazing conditions. Fifty-six bulls were divided across two treatments: supplementation with common mineral salt (C = control 100g/animal/day, n=28), or supplementation with proteinated salt (S = supplemented 500g/animal/day, n=28). The following measurements were taken: body weight and testicular measurements (testicle length, width, and scrotal circumference). Additionally, semen was collected four times during the year (March, July, September and October, 2002) by eletroejaculation, to verify physical (motility and vigor), and morphological aspects of the semen, as well as semen concentration. Data were analyzed using the statistical program SAEG from Federal University of Viçosa (2002). We used descriptive analyses (mean ± standard deviation) for all variables studied, and variance for quantitative parameters (testicular measurements, physical and morphological aspects of the semen). Additionally, when animals within treatment were separated by age, classifying them as young (born 1996 to 1999) or older (born 1988 to 1995), we determined that the change in body weight was greater for the older animals, being a 45.33 kg loss (control treatment), versus a 29.0 kg loss for the supplemented group. For the young animals the change in body weight for the control treatment was a 24.25 kg loss and for the supplemented treatment was a 5.16 kg loss. There was no difference ($P>0.05$) for scrotal circumference among the treatments (C and S). However, there was an increase ($P<0.05$) in left testicular length (LTL) for supplemented bulls starting at the second collection, but was not different in the control treated bulls ($P>0.05$). Right testicular length (RTL) did not change in the supplemented bulls ($P>0.05$). Testicular width increased ($P<0.05$) after the first collection for both treatments (C and S), but there was no difference between treatments ($P>0.05$). The physical aspects of semen (motility and vigor) and semen concentration did not differ ($P>0.05$) between the control and supplemented treatments and between the four collections. For the morphological aspects of the semen in control animals, there was an increase in the larger defects ($P<0.05$) across collections over time. However, the supplemented animals showed no differences across the collections ($P>0.05$). Therefore, supplementation with proteinated salt, under the conditions employed in this study, was able to maintain body weight and normal sperm morphology more effectively than controls, but did not have significant effects on the other reproductive parameters measured in Nelore bulls.

FERTILITY OF LACTATING DAIRY COWS ADMINISTERED BOVINE SOMATOTROPIN DURING HEAT STRESS

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While administration of bovine somatotropin (bST) to lactating dairy cows increases milk production, it can also increase body temperature during heat stress and may therefore compromise fertility. However, bST increases secretion of insulin-like growth factor-I (IGF-I) and culture of bovine embryos with IGF-I blocks the reduction in blastocyst development and induction of apoptosis caused by heat shock. The purpose of this study was to determine effects of bST on reproductive function in lactating dairy cows during heat stress. The experiment was conducted in South Georgia from July to November 2005 using 271 lactating Holstein cows. For first service timed artificial insemination (TAI), cows were presynchronized with two injections of $PGF_{2\alpha}$ given 14 d apart followed by a modified Ovsynch protocol (GnRH given 72 h following $PGF_{2\alpha}$). Pregnancy was diagnosed using ultrasonography on d 29 and reconfirmed by palpation between d 45-80 post-TAI. Non-pregnant cows were resynchronized with the modified Ovsynch protocol and received TAI. Treatment with bST (500 mg; Posilac, Monsanto) started 1 wk prior to the start of Ovsynch and continued at 2-wk intervals. A subset of cows (n=38) were bled for IGF-I profiles immediately prior to the first bST injection, 1 wk later, and at d 35 of bST treatment. Rectal temperatures were taken on d 29 of bST treatment. Pregnancy rates (d 45-80 post-TAI) did not differ between control (n=129) and bST-treated cows (n=142) for first- (15.5% vs 16.9%) or second-service TAI (17.2% vs 15.0%). Milk yield and plasma concentrations of IGF-I were higher for bST-treated cows following the initiation of bST treatment (bST x time interaction, $P<0.01$) and bST increased rectal temperature ($P<0.05$; 39.17°C vs 39.31°C for control vs IGF-I cows, SEM=0.05°C). In conclusion, treatment with bST during heat stress increased IGF-I concentrations and milk production over time and rectal temperature without compromising first- or second-service pregnancy rates. (Research Support: USDA TSTAR grant 2004-34135-14715 and IFAFS grant 2001-52101-11318).

DOES THE ANTI-PROLIFERATIVE ACTION OF OVINE UTERINE SERPIN INVOLVE APOPTOSIS?

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Ovine uterine serpin (OvUS) is the major progesterone-induced protein in the uterus of the pregnant sheep. This protein is a member of the serine proteinase inhibitor superfamily and it has been proposed to down-regulate uterine immune function during pregnancy to protect the fetus. In vitro experiments have shown that both the native and the recombinant (r) form of the protein can inhibit mitogen-induced lymphocyte proliferation and growth of canine primary osteogenic sarcoma cells, mouse lymphoma cells, human prostatic adenocarcinoma cells (PC-3 cell line) and bovine preimplantation embryos. The mechanism by which OvUS inhibits cell proliferation is still unknown. Accordingly, experiments were conducted to test whether rOvUS exerts its anti-proliferative action through apoptosis. In the first experiment, the anti-proliferative effect of rOvUS on PC-3 cells was tested to determine the minimal concentration effective at inhibiting proliferation. Proliferation was inhibited by concentrations of OvUS at 8 μg/ml and higher. The incorporation of [^{3}H]thymidine was 4043, 3998, 3464, 2785, 2827, 2310, and 2332 dpm for 0, 0.5, 2, 8, 32, 125, and 250 μg/ml, respectively. In the second experiment, PC-3 cells were cultured for 48 hours with 50, 100 and 200 μg/ml of rOvUS or ovalbumin. Cells were then fixed and apoptotic cells identified using the TUNEL assay to detect DNA fragmentation. There was no effect of OvUS at any concentration tested on the percent of cells that were apoptotic. Percent apoptosis was 1.3% for control cells, 3.8%, 4.8% and 2% for cells cultured with 50, 100 and 200 μg/ml rOvUS, and 2% for cells cultured with 200 μg/ml ovalbumin. Results confirm that OvUS inhibits proliferation of PC-3 cells and indicate that inhibition does not involve induction of apoptosis. Further experiments are warranted to elucidate the anti-proliferative mechanism of action of OvUS.

DEVELOPMENT OF CULTURE CONDITIONS FOR INVESTIGATING BOVINE BLASTOCYST DEVELOPMENT AND INTERFERON-TAU PRODUCTION FROM DAY 8 TO 11 POST-FERTILIZATION

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Interferon-tau (IFN-τ) is produced by developing bovine conceptuses from the time when blastocysts first form (day 7 post-fertilization) until the conceptus begins to attach to the uterine lining (day 21 of pregnancy). At day 16 of pregnancy, sufficient quantities of IFN-τ must exist within the uterus to limit the pulsatile release of prostaglandin F2α, which permits continued luteal function and perpetuation of a pregnant state. There is substantial evidence that the uterus contributes to the successful development of the conceptus and to promoting IFN-τ during early pregnancy. The overall goal of this research is to identify uterine-derived factors that influence IFN-τ production in bovine conceptuses and to determine if these factors may be used to promote pregnancy success in dairy cattle. The aim of the present project is to determine the culture conditions that are required to permit continued blastocyst survival and IFN-τ production from day 8 to 11 in bovine embryos. In the first study, our objective was to determine if medium type and oxygen concentration affects blastocyst survival and IFN-τ production. Bovine embryos were produced by using in vitro maturation, fertilization, and development procedures. Blastocysts obtained on day 8 post-fertilization were cultured individually in 30 µL drops in one of two culture media (M-199 or KSOM with 5% FBS + Glutamine and Na Pyruvate) and in a 20% or 5% oxygen environment. On day 11 post-fertilization, embryos were removed from culture, fixed, and the individual medium samples were collected. Total cell number and the percent of cells undergoing apoptosis were determined by Hoescht staining and TUNEL analysis, respectively. Antiviral assays were completed on the individual medium samples to determine the amount of IFN-τ produced from each embryo over the 72 h incubation period. Preliminary results indicate that embryo survival and detectable IFN-τ production was best realized when culturing bovine blastocysts in M-199 at 5% oxygen concentration. The upcoming pilot study will investigate the ideal serum concentration required to sustain embryo development from day 8 to 11 post-fertilization. After these pilot studies are completed, our focus will turn to examining the effects of two growth factors, fibroblast growth factor-2 and granulocyte-macrophage colony-stimulating-factor, on embryo development and IFN-τ expression from in vitro- and in vivo-derived bovine embryos. Future studies will investigate schemes to enhance embryo development and/or IFN-τsecretion through treatment with particular growth factors and examine whether pregnancy success can be improved in embryos receiving these embryotrophic factors.

EFFECT OF THE ADDITION OF INSULIN-LIKE GROWTH FACTOR-1 TO EMBRYO CULTURE MEDIUM ON PREGNANCY RATES FOLLOWING TIMED EMBRYO TRANSFER IN LACTATING DAIRY COWS

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Addition of insulin-like growth factor-1 (IGF-1) to embryo culture medium increases pregnancy rates following transfer of in vitro produced embryos to heat stressed, lactating dairy cows (Block et al., J. Anim. Sci. 81:1590, 2003). The objective of the present study was to determine whether the effect of IGF-1 on embryo survival was a general effect or one specific to heat stress. An embryo transfer field trial was conducted between March 2005 and January 2006 at 4 different locations. Embryos were produced in vitro using oocytes collected from abbatoir-derived ovaries. After fertilization, presumptive zygotes were cultured in the presence or absence of 100 ng/mL IGF-1. Cows were synchronized for timed embryo transfer using the OvSynch protocol (3 locations) or 2 injections of prostaglandin $F_{2\alpha}$ (25 mg, i.m.) 14 days apart (1 location). A total of 287 primiparous and multiparous, lactating cows were selected as recipients based on the presence of a corpus luteum. Grade 1 embryos were selected on d 7 after fertilization and randomly transferred to recipients that were at d 7 after anticipated ovulation. Pregnancy rate was diagnosed by ultrasonography at d 27-32 and by rectal palpation at d 41-49. The experiment was replicated 20 times with 6-28 recipients per replicate. Transfers were divided into two seasons, cool (Jan., Mar., April, Nov., Dec.) and hot (July, Aug., Sep.). There was a significant IGF-1 x season interaction for pregnancy rate at both d 27-32 and d 41-49 ($p<0.01$). Addition of IGF-1 to embryo culture significantly increased pregnancy rate during the hot season (d 27-32: 34/69 = 49.3% vs. 15/71=21.1% and d 41-49: 28/67=41.8% vs. 13/71=18.3%, respectively), but not during the cool season (d 27-32: 19/70 = 27.1% vs. 23/66=34.9% and d 41-49: 16/73=21.9% vs. 21/74=28.4%, respectively). Results indicate that IGF-1 can be added to bovine embryo culture to increase pregnancy rate in lactating dairy cow recipients during heat stress but there is no advantage in the absence of heat stress. USDA-TSTAR 2004-34135-14715 and BARD US-3551-04

EFFECTS OF SELENIUM (SE) SOURCES ON IMMUNITY, HEALTH, PREGNANCY AND MILK PRODUCTION OF DAIRY COWS IN FLORIDA DURING SUMMER

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Objectives were to evaluate effects of organic Se on postpartum (pp) innate and adaptive immunity, pp uterine health, pregnancy rates (PR) at the first two pp services and milk yield in summer. Cows were supplemented with Se (Se-yeast [SY; Sel-Plex®, Alltech; n=289] or sodium selenite [SS; n=285]) at 0.44 ppm in dry matter from -23 ± 8 days (d) pp to parturition and 0.3 ppm from parturition to $\geq$81 dpp. Innate immunity was determined by phagocytic and oxidative burst capacity of neutrophils in whole blood using a dual color flow cytometric method (Silvestre et al, AMCB 2005). Samples were collected from multi-and primiparous (n=10/diet, respectively) cows at -26 (day of initiating of SY), 0, 7, 14, 21 and 37 dpp for neutrophil function and for Se measurements in plasma. Adaptive immunity was monitored with anti-IgG to Ovalbumin (Ovalb) following vaccination with Ovalb antigen (1 mg [s.c.]) dissolved in an *E. coli* J5 endotoxemia preventive vaccine at -60 and -22 ± 6 dpp (day of initiating of SY [n=38] and SS [n=47] diets) and again at parturition (day 0) with Ovalb dissolved in PBS with Quil-A adjuvant. Serum samples were collected on days of immunization and at 21 and 42 dpp. Anti-IgG to Ovalb was measured via an ELISA assay with absorbance (OD) read at 405 and 650 nm. Rectal temperature (RT) was recorded daily for the first 10 dpp and vaginoscopies made at 5 and 10 dpp. Cows within diet were assigned randomly to two reproductive programs (RPs; Presynch-Ovsynch vs CIDR-Ovsynch [i.e., Ovsynch begins 3 d after withdrawal of a 7 d-CIDR]). After Timed (T) AI, all cows were resynchronized for a 2nd service with Ovsynch at 20-23 d after 1st service and pregnancy diagnosis at 27-30 d via ultrasography. Pregnancies were reconfirmed by rectal palpation at 55 d. Cows in estrus following Presynchs were AI up to the 2nd TAI service. Strategic blood sampling determined anovulatory status at Ovsynch and ovulatory response after TAI to 1st service. PR for 2nd service was made at ~42 dpp by rectal palpation. Milk composition, yield and somatic cell count were obtained monthly from DHIA records (Raleigh, NC). Plasma Se increased in SY fed cows ($0.087 > 0.069 \pm 0.004$ µg/ml; $P<0.01$). Percentage of gated neutrophils that phagocytized *E. coli* and underwent oxidative burst did not differ between diet groups at -26 dpp (44.6 ± 4.6%). For subsequent samples, a diet*parity*day interaction was detected ($P<0.05$): SY improved neutrophil function at parturition in multiparous cows ($42 \pm 6.14\% > 24.3 \pm 7.2\%$) and at 7, 14 and 37 dpp in primiparous cows ($53.9 > 30.7$, $58.6 > 41.9$, $53.4 > 34.8$%, respectively; pooled SE=6.8%). Anti-IgG to Ovalb did not differ between diets at -60 and -22 dpp (0.18 ± 0.01 and 0.97 ± 0.04 OD). Although Anti-IgG to Ovalb concentration did not differ between diet groups for primiparous cows (1.40 ± 0.08 OD), concentrations were higher in SY cows at 21 and 42 dpp ($1.91 \pm 0.1 > 1.24 \pm 0.07$, $1.44 \pm 0.7 > 0.99 \pm 0.07$ OD, respectively; $P<0.01$). SY reduced frequency of multiparous cows with $\geq$1 event of fever (RT $\geq 39.5^{0}$C; 13.3% vs 25.5%; $P<0.05$) but not in 1st parity cows (40.5%). Vaginoscopy scores for clear (47% vs 35%), mucupurulent (43% vs 48%) and purulent (9% vs 17%) discharges were affected by SY and SS, respectively ($P<0.05$). Diets and RPs did not alter frequencies of retained fetal membrane (9.7%), displaced abomasum (2.44%), ketosis (5.0%), mastitis (14.4%), anovulation (17.7%) and synchronized ovulation after TAI (82.5%). Diets and RPs failed to alter 1st service PR at ~d 30 (SY, 24.9% vs SS, 23.6%) or pregnancy losses between ~d 30 and ~d 55 (SY, 39.3% vs SS, 37.1%). SY improved 2nd service PR (17% vs 11.3%; $P<0.05$). SY cows produced more 3.5% fat corrected milk (FCM; 36.2 vs 35.3 kg/d; $P<0.05$) but source of Se did not alter milk somatic cells counts (291,618 /mL), In summary, feeding organic Selenium as Se-yeast beginning at prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition and humoral immunoresponsiveness in multiparous cows during early lactation. Furthermore, feeding organic Se to periparturient cows improved uterine health, FCM and 2nd service PR, but not 1st service PR.

HEAT SHOCK OF FROZEN/THAWED EJACULATED SPERMATOZOA AFFECTS FERTILIZATION AND SUBSEQUENT EMBRYONIC DEVELOPMENT

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It has been well documented that heat shock can compromise the function of spermatagonia, spermatocytes, oocytes and preimplantation embryos. It is not known, however, whether heat shock can damage ejaculated sperm in a manner that would affect fertilization and development of embryos formed from heat-shocked sperm. Here, it was hypothesized that heat shock (40°C for 4 h) of frozen/thawed ejaculated spermatozoa would lead to a reduction in fertilization and embryo development. Extended semen was thawed, subjected to Percoll gradient purification to obtain motile spermatozoa, diluted in Sp-TALP medium to 20 x 10^6 spermatozoa/ml, and incubated at 38.5°C or 40° C in air for 4 h using a water bath. Additional semen was thawed to prepare an unincubated spermatozoa control. In this case, semen was thawed, subjected to Percoll purification, and diluted to 20 x 10^6 spermatozoa/ml at a time coincident with the end of the incubation period for heat shock. Following treatment, sperm was used to fertilize oocytes that had been matured for 22 h. Treatments were fertilization with non-incubated sperm, fertilization with sperm incubated at 38.5°C, fertilization with sperm incubated at 40°C, and a parthenogenesis control (incubation of oocytes in fertilization medium without sperm). Oocytes were fertilized in groups of 30 with 500,000 spermatozoa/well. Presumptive zygotes and oocytes were placed in groups of 10 in 25 µL microdrops of KSOM-BE2 and cultured at 38.5ºC in an humidified atmosphere of 5% CO_2, 5% O_2 and the balance nitrogen. Cleavage was accessed on Day 3 after insemination and blastocyst development on Day 8 after insemination. The experiment was replicated 6 times, with a separate bull used for each replicate. Exposure to 40°C tended to reduce cleavage rate (P=0.0995) as compared to 38.5°C. Cleavage rates (least-squares means ± SEM) were 80.0 ± 4.3% (non-incubated), 63.2 ± 4.3% (38.5°C), 52.9 ± 4.3% (40°C) and 42.9 ± 4.9% (parthenogenesis). Cleavage rate was higher (P=0.0009) for oocytes incubated with non-incubated sperm as compared to incubated sperm but there was no significant difference in cleavage rates between 38.5°C and 40°C (P>0.05). The proportions of oocytes that became blastocysts were 28.2 ± 3.1% (non-incubated), 21.1 ± 3.1% (38.5ºC), 4.0 ± 3.1% (40ºC) and 4.3 ± 3.5% (parthenogenesis). Development was higher (P=0.001) for oocytes incubated with non-incubated sperm as compared to incubated sperm and higher for embryos formed from sperm incubated at 38.5°C as compared to those at 40°C (P=0.0015). Similar treatment effects were found for percent of cleaved embryos becoming blastocysts (data not shown). In conclusion, exposure of sperm to heat shock prior to fertilization had a detrimental effect on cleavage rate and subsequent embryonic development. The mechanism of action by which heat shock of sperm affects developmental competence of embryos formed from that sperm is unclear. One possibility is that this may be due to increased apoptosis in the developing embryo as was recently seen in bovine embryos produced from spermatozoa exposed to radiation. Another possibility is that most of the embryos formed in the 40ºC group were parthenotes. Further experiments are required to resolve this issue.

THE EFFECT OF TRANSVAGINAL ASPIRATION OF SMALL AND MEDIUM SIZED FOLLICLES ON SERUM PROGESTERONE, FSH AND LH CONCENTRATIONS IN MARES

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Equine embryo and oocyte transfer programs depend upon synchronization of donor and recipients. Reliable methods to induce a CL in recipient mares are critical. Previously it was shown that following aspiration of preovulatory follicles, serum progesterone was elevated 3-5 days after aspiration. However, it was unclear if aspiration of smaller, non-periovulatory follicles responded to follicular aspiration similarly. We hypothesized that aspiration of follicles (<35mm) during estrus results in the formation of a hormonally functional CL. Our objectives were to determine the serum concentrations of progesterone, LH and FSH in estrus mares undergoing aspiration of follicles <35mm. Seven light breed mares were determined to be in estrus based on transrectal palpation and ultrasound. Follicles were grouped as A (<25mm), B (26-30mm), and C (31-34mm) based upon their size at aspiration. One follicle from each group was aspirated in 7 mares, yielding 21 aspirations. All aspirations were performed during estrus using ultrasound guided transvaginal technique. Jugular blood samples were collected on days –1, 0, 1, 2, 5 and 8 after aspiration to determine hormone concentrations. At the same time, CL formation was monitored using transrectal ultrasonography.
Plasma LH concentrations were determined by radioimmunoassay (RIA) and plasma FSH concentrations were measured by a heterologous double-antibody RIA validated in the laboratory (Roser and Hughes, 1991). Progesterone was measured using a commercial RIA kit (Coat-a-Count Kit, Diagnostic Products, Los Angles, CA). Luteal tissue was considered present if progesterone concentration was greater than 1ng/mL. Mares spontaneously ovulating during the experiment were excluded. Data was analyzed by Kruskal-Wallis One-Way Nonparametric AOV. Significance was set at $p<0.05$.
Aspirated follicles in Group B resulted in CL formation in 88% of mares, compared to 0% in Group A ($p<0.05$). CL formation showed no difference between Groups B and C (88% v 100%). Progesterone concentrations were > 1ng/mL by day 5 in 80% of the mares and by day 8 in the remaining 20%, that formed a CL (Group B and C). LH concentrations increased significantly higher after aspiration in Groups B and C compared to Group A ($p<0.05$). There was no difference in FSH concentrations between groups ($p=0.4$).
These results suggest that a hormonally functional CL can be reliably induced in estrous mares by aspiration of follicles >25mm and that aspiration of these follicles induces an LH surge. The mechanism for an increase in LH concentrations after follicular aspiration is unknown, but is not different from observed increases in LH, 1-2 days after spontaneous ovulations.

VITRIFICATION OF IN VITRO PRODUCED BOVINE EMBRYOS

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The advancement of in vitro production of bovine embryos, and other artificial reproductive techniques, such as cloning, gives producers more flexibility in reproductive management and maintaining genetic diversity of their herds. However, a successful method for long-term storage of these embryos is much needed. Cryopreservation methods currently available are not yet efficient for embryos produced by these technologies. Factors affecting the efficiency of cryopreservation programs are the origin and stage of development of the embryos, along with the cryoprotectant and method of freezing used. Using the slow-freeze method with in vivo produced embryos yields consistently good results, and is well accepted by the industry. In vitro produced embryos are known to have a higher sensitivity to chilling and freezing than in vivo produced embryos, resulting in lower viability post-thaw. Vitrification is a newer technology in which the cryoprotectant and embryo is solidified due to an extreme increase of viscosity during cooling. While the fast cooling rate of vitrification bypasses ice crystal formation, which typically occurs from +15 to -5°C, it does not eliminate other negative effects, such as osmotic injury and changes to the cytoskeleton. The high concentration of cryoprotectant in vitrification media, while required, can result in irreversible injury to the cytoskeleton. Several factors can affect the efficiencies of this technology including culture medium, temperature, cryoprotectants and additives. An overview of our proposed vitrification experiments will be discussed.

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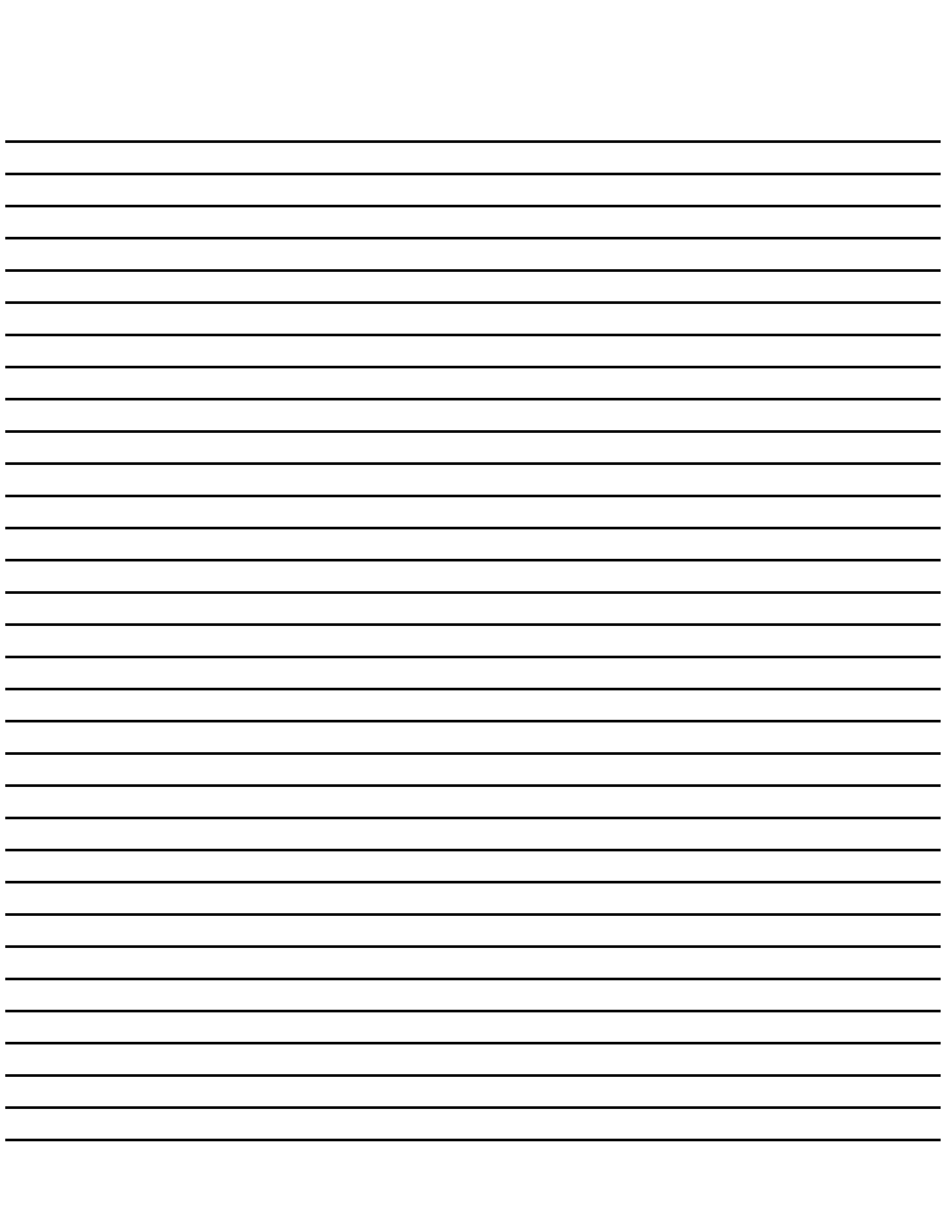

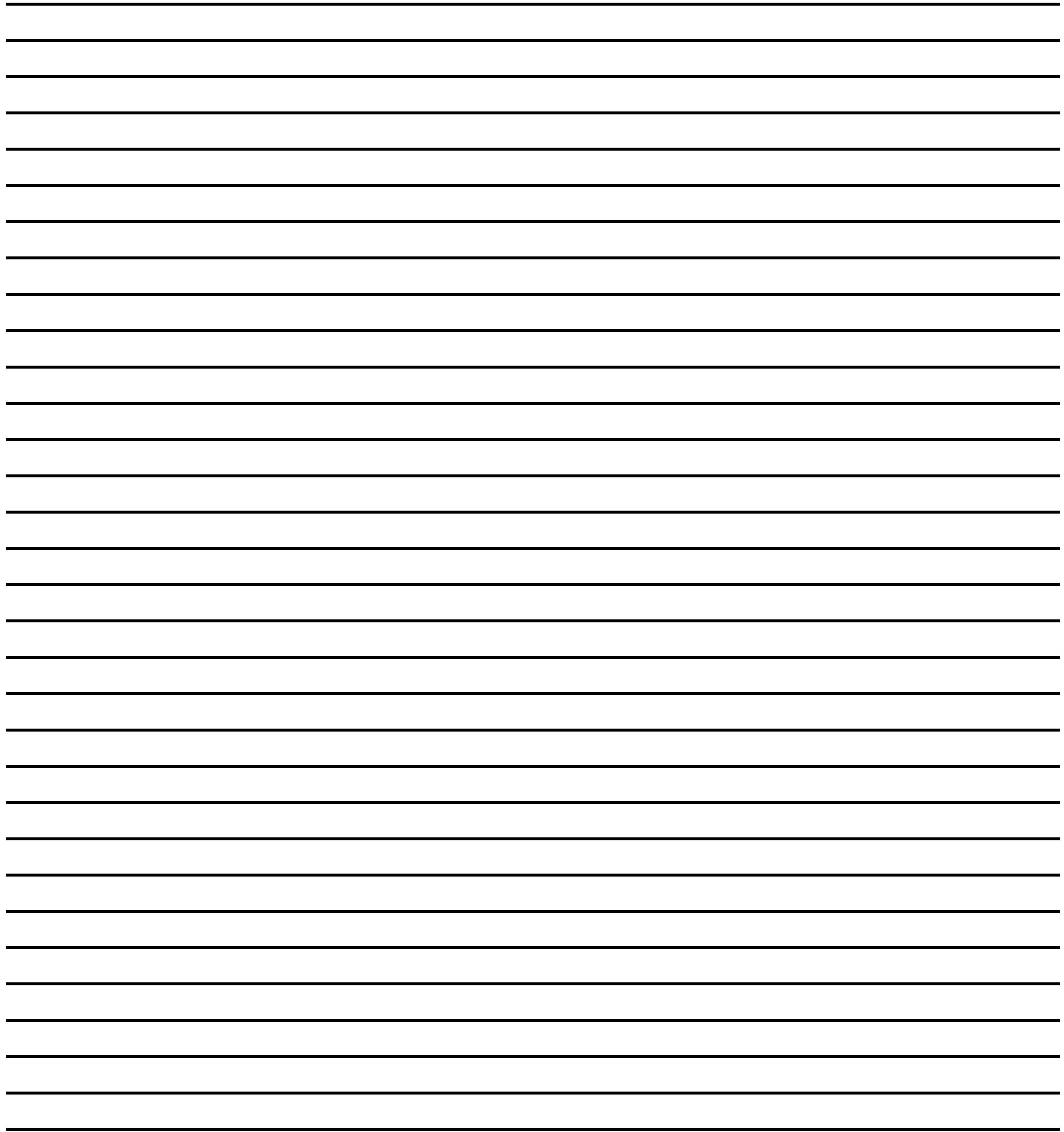

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