(LPN), intermediate (IPN), and a high (HPN) plane of nutrition based on the CP and ME of diets. Birds and feed were weighed at the end of the starter (35 d), grower (63 d), and finisher (84 d) periods to obtain body weight (BW), feed intake (FI), and feed conversion (FC). At 84d, 35 birds per treatment were randomly selected and processed to evaluate carcass composition. The weights of New York dressed (NYD), ready to cook (RTC), and fat pad (FP) were obtained and yields calculated as a percentage from live BW. A significant main effect of genotype for BW was found at 35, 63, and 84 d where both CG birds had similar but significantly heavier BW than NG birds. A significant effect of diet was observed at 35 and 84 d, where birds fed HPN diets had significantly heavier BW weights than those fed IPN and LPN diets. At all ages, CG1 and CG2 had similar but significantly lower FC compared to NG. However, no significant differences were observed for FI. There was a significant genotype and plane of nutrition interaction where birds of the CG1 fed HPN and IPN diets and CG2 fed IPN diet had significantly heavier NYD and RTC weights. Carcass RTC yields of CG were significantly higher compared to NG birds. Guineas of the NG had significantly lower percentage of FP than both CG evaluated. Birds raised under a HPN and IPN regime had significantly FP yield than those raised under a LPN regime. This investigation confirms that genetic selection has made significant improvements on performance and carcass traits of guinea broilers. The results showed that improvements in performance traits and processing yields may be obtained when CG are raised under a HPN regime.

Key Words: Guinea, Genotype, Nutrition

Physiology & Endocrinology - Livestock and Poultry: Reproductive Physiology

W199 Influence of post-AI nutrition on blood urea nitrogen, progesterone, and pregnancy. G. A. Perry*, B. L. Perry, J. R. Nelson, and J. A. Walker, *South Dakota State University*, *Brookings*.

Research has shown that changes in nutrition can have an effect on reproductive performance. Our objective was to determine the effect of post-AI nutrition on BCS, blood urea nitrogen (BUN), progesterone, and pregnancy rates. Forage-developed Angus-cross heifers (n = 336) were synchronized with the Select Synch+CIDR protocol (d -7 100 µg GnRH and CIDR; d 0 25 mg PG and removal of CIDR; Estrus detected for 72 h and heifers bred 12 h after detection in estrus; heifers not in estrus were bred with an injection of GnRH at 72 h). Each breeding period was equally divided into three treatments: 1) heifers returned to feedlot (LOT), 2) heifers were moved to pasture (PASTURE), or 3) heifers were moved to pasture and supplemented with 2.22 kg/hd/d of dried distillers grains plus solubles (SUPP). Blood samples were collected on d -7, 0, 2, 14 and 42 (pregnancy determination; analyzed by repeated measures). BCS were determined on d -7 and 42. All heifers were in similar ($P = 0.78$) BCS (5.4 ± 0.05) on d -7, but on d 42 SUPP (5.9 ± 0.04) were in greater condition ($P < 0.01$) than LOT (5.8 ± 0.04) which were in greater condition ($P < 0.01$) than PASTURE (5.4 ± 0.04). All treatments had similar ($P > 0.14$) BUN concentrations on d -7 (129 ± 1), but on d 2, 14 and 42 SUPP had greater ($P < 0.01$) BUN concentrations compared to both LOT and PASTURE. There was no difference in BUN concentrations between pregnant and open heifers ($P = 0.37$). Progesterone concentrations were similar among all heifers ($P \geq 0.05$) on d 0 and 2. SUPP had greater progesterone on d 14 ($P = 0.02$) compared to LOT, and on d 14 and 42 PASTURE had greater progesterone ($P < 0.02$) compared to LOT. Progesterone was similar ($P > 0.16$) for open and pregnant heifers on d 0 and 2, but greater ($P < 0.04$) in pregnant heifers on d 14 and 42. There was no difference among treatments in pregnancy rates ($P > 0.64$; 57, 56, and 59% for SUPP, LOT, and PASTURE; analyzed by chi-square). In summary, supplementing forage-developed heifers after insemination increased BCS and BUN concentrations, but had no effect on pregnancy rates.

Key Words: Heifers, Fertility, Post-AI Nutrition

W200 Effect of dietary ω-3 polyunsaturated fatty acid supplementation on hormone and metabolite concentrations and corpus luteum size in beef heifers. S. Childs*[1,2], J. M. Sreenan[1], A. A. Hennessy[3], C. Stanton[3], M. G. Diskin[1], and D. A. Kenny[2], [1]*Teagasc Animal Production Research Centre*, *Athenry, Co. Galway, Ireland*, [2]*University College*, *Dublin, Ireland*, [3]*Teagasc Moorepark Food Research Centre*, *Co. Cork, Ireland*.

Supplementation of cattle diets with fishoil has been reported to improve fertility. Though the mechanisms involved remain unclear, it is thought that constituent ω-3 polyunsaturated fatty acids (ω-3 PUFA) eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids may mediate this effect. The objective of this study was to examine the effect of level of a high ω-3 PUFA product on a number of important reproductive variables. Heifers (n=40) were randomly assigned to a concentrate and straw (80:20) based ration supplemented with one of four levels of a high ω-3 PUFA product to provide on a DM basis: (1) 0g (**C**); (2) 62g (**T2**); (3) 129g (**T3**) or (4) 273 g (**T4**) of EPA and DHA combined. Diets were offered for 45 days and were isolipid and isonitrogenous. Heifers were oestrous-synchronised and plasma samples were collected to determine progesterone (P_4) and oestradiol (E_2) concentrations on day of oestrus (0) and on days 4, 7, 10, 14 and 16 post oestrus. Corpus luteum (CL) size was measured on day 7. Samples for fatty acids (FA) and cholesterol analysis were collected on day 16. FA methyl esters were separated by gas chromatography. P_4 and E_2 were measured by RIA. Data were analysed using repeated measures ANOVA. There was a positive linear effect of dietary ω-3 PUFA on plasma EPA ($P<0.0001$) and both positive linear ($P<0.01$) and quadratic ($P<0.05$) components to the effect on plasma DHA. Plasma cholesterol was similar for C, T2 and T3, and higher ($P<0.05$) for T4 compared with C or T2. There was no effect of treatment ($P>0.05$) on E_2. CL diameter was greater ($P<0.05$) on T3 and T4 than C or T2. On day 14, P_4 concentrations were higher on T4 than on C and T2 ($P>0.01$) but did not differ between other treatment comparisons ($P>0.05$). Omega-3 PUFA supplementation may increase P_4 concentrations around the critical period of maternal recognition of pregnancy. This increase may be mediated through increased substrate availability and/or CL size.

Key Words: Omega-3 PUFA, Reproductive Hormones, Fertility

W201 Effect of level of dietary supplementation on concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in selected tissues in cattle. S. Childs*[1,2], J. M. Sreenan[1], A. A. Hennessy[3], C. Stanton[3], and D. A. Kenny[2], [1]*Teagasc Animal Production Research Centre*, *Athenry, Co. Galway, Ireland*, [2]*University College*, *Dublin, Ireland*, [3]*Teagasc Moorepark Food Research Centre*, *Co Cork, Ireland*.

Increased intake of omega-3 polyunsaturated fatty acids (ω-3 PUFA) has been suggested to improve fertility in cattle. However, as long-chain PUFA are extensively hydrogenated in the rumen, this often results in poor transfer from diet to tissue. The objective of this study was to examine the effect of level of dietary ω-3 PUFA supplementation on concentrations of both EPA and DHA in blood plasma (PL), rumen fluid (RF), follicular fluid (FF) and uterine endometrial tissue (ET) in cattle. Heifers (n=40) were randomly assigned to a concentrate and straw (80:20) based ration supplemented with one of four levels of a partially rumen-protected high ω-3 PUFA product to provide on a DM basis: : (1) 0g (**C**); (2) 62g (**T2**); (3) 129g (**T3**) or (4) 273 g (**T4**) of EPA and DHA combined. Diets were offered on an individual basis and were isolipid and isonitrogenous. Heifers were slaughtered after 45 days on experiment. Blood samples for PL were collected on day 44 and RF, FF and ET samples were collected immediately post-slaughter for fatty acid analysis. Data were analyzed using ANOVA and multiple regression analyses. There was a positive linear effect of treatment on concentrations of EPA in PL ($P<0.0001$), FF ($P<0.05$) and ET ($P<0.0001$) but no effect in RF ($P>0.05$). There was a positive linear ($P<0.01$) effect of treatment on DHA concentrations in RF with a tendency towards a quadratic component ($P=0.07$). The effect of diet on PL and FF concentrations of DHA was positive with both linear ($P<0.01$) and quadratic ($P<0.05$) components while the effect on ET concentration was both positive and linear in direction ($P<0.05$). Blood plasma concentrations of EPA ($R^2=0.69$) and DHA ($R^2=0.28$) were the best predictors of their respective concentrations in ET. Tissue concentrations of ω-3 PUFA can be manipulated through rumen protection of dietary supplements. Blood plasma concentration may be a useful predictor of PUFA concentration in reproductive tissues.

Key Words: Omega-3 PUFA, Rumen Protection, Reproductive Tissue

W202 Nutritional and genetic effects on the follicular growth of the Nelore-Hereford heifers. J. O. J. Barcellos*, E. R. Prates, J. López, and J. Braccini, *Federal University of Rio Grande do Sul*, *Porto Alegre- RS - Brasil*.

The objective of this study was to evaluate the effects of growth rate during the post-weaning phase to puberty on the follicular growth of the two hundred beef heifers Nellore-Hereford crosses. The heifers were weaned in the six months old and allotted in the factorial experimental design 4 (weight gain daily) × 5 (crossbreed degree-CX) and treatments were: 50 heifers (25%N-75%H; 37.5%N-63.5%H; 43.7-56.3%H; 50%N-50%H and 75%N-25%H) submitted to an average growth rate (AGD) of 0.500 kg/day (G500); 50 heifers (25%N-75%H; 37.5%N-63.5%H; 43.7-56.3%H; 50%N-50%H and 75%N-25%H) with an ADG of 0.750 kg/day (G750); 50 heifers (25%N-75%H; 37.5%N-63.5%H; 43.7-56.3%H; 50%N-50%H and 75%N-25%H) with an ADG of 1.000 kg/day (G1000) and 50 heifers (25%N-75%H; 37.5%N-63.5%H; 43.7-56.3%H; 50%N-50%H and 75%N-25%H) with an ADG of 1.250 kg/day (G1250). The experimental diets were adjusted to achieve at puberty an 12-13 months of age. The diameter of the large follicle (DLF- mm) and the follicular area total (FA- mm2) at the 10, 11 and 12 months of age by real-time ovarian ultrasonography Aloka SD500 model, in the total heifers were evaluated. The data were analyzed by ANOVA and stepwise regression. The average age at puberty was at 338 ± 22 days. The general equation by DLF at 10 months were DLF=8.642*ADG-3.3615 (r2=0.70). At the 10 months, the results showed an interaction ($P<0.01$) between the ADGxCX with the higher effect of the ADG on the DLF in the heifers with minus of the 50%N. The effects of the nutritional levels were similar at 11 and 12 months ($P>0.06$). However, the G1250-75N showed higher variations ($P<0.05$) on the DLF from the 10 to 12 months (4.5mm × 10.6mm) than heifers 25, 35.5, 43.7 and 50% ones. The heifers 25, 35.5 and 43.7%N showed higher AF at 10, 11 and 12 months age and the effect were very important in the G1000 and G1250 ($P<0.01$). Therefore, the weight gain posweaning have the effect important on the follicular growth to puberty early in crossbreed heifers.

Key Words: Puberty, Beef Cattle, Reproduction

W203 Levels of serum progesterone in creole cows with and without corpus luteum treated with CIDR®, progesterone, β-estriadiol and $PGF_{2\alpha}$. J. P. Zarate Martinez*, J. A. Ramirez Godinez, and F. A. Rodriguez Almeida, *Universidad Autonoma de Chihuahua*, *Chihuahua, Chih. Mexico*.

Experiments were conducted to study the serum progesterone (P_4) levels in cows with ($P_4 \geq 1$ ng/mL) and without corpus luteum (CL), treated with two hormone protocols that used an intravaginal CIDR® device. Cows were assigned randomly to one of two treatments (T). T1 (n=14) received a CIDR® with 1.9 g of P_4 + IM injection of 1 mg β-estriadiol and 50 mg of P_4, and CIDR® was withdrawn on day 7, when an IM injection of 30 mg of $PGF_{2\alpha}$, and on day 8 1 mg IM of β-estriadiol was administered; T2 (n=13) was similar but IM injection contained 1 mg of β-estriadiol without P_4. To determine the concentrations of P_4, blood samples were taken on days (D) 0, 1, 2, 7, 8, and 9 after application of CIDR®. Serum concentrations were analyzed with PROC MIXED of SAS, adjusting a model with main effects of CL, T, D and their interactions, and random effects of cows within T*CL. Seventy-five percent of cows exhibited estrus. There were no differences ($P>0.05$) for P_4 concentrations between T1 (5.63±0.497 ng/mL) vs T2 (4.51±0.486 ng/mL). The presence of a CL at the beginning of hormone application protocols was not significant ($P>0.05$) for the average concentrations of P_4 in cows with CL (5.85±0.36 ng/mL) and cows without CL (4.31±0.59 ng/mL) during the days of treatment. The interaction T*D showed numerical differences in the P_4 concentrations. There were differences between T1 (10.90±0.921 ng/mL) and T2 (7.46±1.054 ng/mL) on day 1 and this trend continued until CIDR® was withdrawn; however differences were not significant ($p>0.05$). Higher values of P_4 concentrations for T1 cows, which received an IM injection of 50 mg of P_4 besides the CIDR®, than for T2 cows, which did not received P_4 injection, were as expected; however more observations are needed to confirm findings.

Key Words: Creole Cows, CIDR, Corpus Luteum

W204 Effect of progestin treatment on formation of persistent follicles in beef heifers. M. E. Heaton*, J. A. Atkins, J. F. Bader, C. L. Johnson, and M. F. Smith, *University of Missouri*, *Columbia*.

Effective estrous synchronization protocols frequently utilize progestins (melengestrol acetate [MGA] and Controlled Internal Drug Release [CIDR] inserts) to synchronize estrus. Previous research demonstrated that long-term treatment with MGA, in the absence of a corpus luteum, caused formation of persistent follicles and resulted in low fertility. The specific aims of this project were to determine if the presence of a new or used CIDR, in heifers without a corpus luteum, would induce the formation of persistent follicles and to compare the pattern of serum concentrations of progesterone in heifers treated with a new or used CIDR to luteal phase concentrations of progesterone (P4) in non-treated heifers. Normally cycling heifers were allocated by age, weight, and breed into four treatment groups: Control (n=8), MGA (n=4; 0.5 lbs-1hd-1day), new CIDR (n=7; 1.38 g P4), and used CIDR (n=8; new CIDR's previously inserted into cows for 7 d). Progestin treatment (MGA or CIDR) began on d 4 post-estrus and PG was injected on d 6 to induce luteolysis (d 0 = estrus). MGA or CIDR treatment continued for 14 d and length of a follicular wave was defined as the interval from follicular recruitment to ovulation or initiation of a new wave. Length of the first follicular wave (d) was 10.9^{a}, 18.0^{b}, 17.1^{b}, and 16.9^{b} ($^{ab}P \leq .05$) and maximum diameter (mm) of the dominant follicle was 14.4^{c}, 18.8^{d}, 16.0^{c}, and 18.5^{d} (Control, MGA, new CIDR, and used CIDR, respectively; $^{cd}P \leq .06$). Dominant follicle diameter was greater ($P \leq .05$) in the used CIDR group compared to the new CIDR group after d 10 of treatment but similar to the MGA group. Serum concentrations of progesterone in the new and used CIDR groups were similar ($P \geq .05$) throughout the 14 d treatment period but lower than in the control group. In summary, treatment with a new or used CIDR induced formation of persistent follicles in beef heifers and there was no difference in serum concentrations of progesterone between the two CIDR groups.

Key Words: Progesterone, Persistent Follicle, Estrous Synchronization

W205 Relationships between cortisol concentrations and cow temperament with calf exit velocity from 3 weeks of age through weaning. N. C. Burdick*[1], R. D. Randel[2], J. P. Banta[2], D. A. Neuendorff[2], J. C. White[2], J. G. Lyons[3], T. H. Welsh, Jr.[3], R. C. Vann[4], and J. C. Laurenz[1], [1]*Texas A&M University-Kingsville*, *Kingsville*, [2]*Texas A&M University Agricultural Research and Extension Center*, *Overton*, [3]*Texas A&M University*, *College Station*, [4]*Mississippi State University*, *Raymond*.

The relationship between cortisol concentrations and cow temperament with exit velocity (EV) in Brahman calves from 3 weeks of age through weaning was assessed. Blood samples were collected from calves (n=116) and their dams on d21-to-24 after birth and from the calves at weaning. Serum concentrations of cortisol (CS) were determined by RIA. Calf EV was determined on d21-to-24 of age and at 28-d intervals until weaning (d173±2) as a measure of temperament. Calves were ranked based on their EV on d21-to-24 (EV Rank) with calves 1 SD slower than the mean ranked 1 (calm; n=17), calves 1 SD faster than the mean ranked 3 (temperamental; n=19), and remaining calves ranked 2 (intermediate; n=80). A subjective measurement of cow temperament was assessed by individual observer with cows being ranked as calm, intermediate, or temperamental. Temperamental cows had greater concentrations of CS (7.2±0.7; $P<0.01$) than calm (3.9±0.4) and intermediate cows (4.2±0.3). Cow CS concentrations were not associated with calf CS concentrations early in life (d21-to-24; $P=0.91$) or at weaning ($P=0.63$). Cow temperament did not affect calf EV from d21-to-24 to weaning ($P=0.39$). However, calf EV was affected by calf EV Rank on d21-to-24, with temperamental calves having a greater EV (3.0±0.12; $P<0.01$) at all ages when compared with calm (1.7±0.2) and intermediate calves (2.1±0.1). Also, calf CS concentrations on d21-to-24 were associated with calf EV Rank on d21-to-24, with temperamental calves having greater concentrations of CS (5.2±0.8; $P<0.01$) when compared to calm (3.0±0.4) or intermediate calves (3.6±0.2). Calf CS at weaning, however, was not related to calf EV Rank on d21-to-24 ($P=0.22$), although temperamental calves had numerically greater concentrations than calm or intermediate calves. Collectively, these data suggest that although cow temperament is related to cow CS concentrations, and calf EV is associated with calf CS, calf EV is not affected by cow temperament. Calm and intermediate calf EV increased through d67 of age before reaching a plateau, while the EV of temperamental calves displayed little change over time.

Key Words: Temperament, Cortisol, Calves

W206 Microbial flora of normal and abnormal cervical mucous discharge associated with reproductive performance of cows and heifers in estrus. A. Ata, H. Turutoglu, M. Kale, M. S. Gulay*, and F. Pehlivanoglu, *Mehmet Akif Ersoy University*, *Burdur, Turkey*.

The aim of the present study was to describe whether abnormal cervical mucus discharge (A-CMD) or pathogens such as aerobic bacteria and fungi in cervical mucus discharge (CMD) have effects on reproductive performance (RP) of cows and heifers in estrus. For this purpose, CMD of 222 animals in estrus were evaluated visually before artificial insemination (AI). Animals having clear discharges (68 cows, 38 heifers) with normal viscosity and without bad odor were grouped as normal cervical mucous discharge (N-CMD) group. The other animals (84 cows, 32 heifers) were grouped as A-CMD group. CMD samples were submitted to cultural examination for *Campylobacter spp.*, *Brucella spp.*, aerobic bacteria and fungi. Microorganisms isolated from samples were divided three groups as uterine pathogens (UP), potential uterine pathogens (PUP) or opportunistic uterine pathogens (OUP). Presence of PUP was associated with A-CMD for both cows ($P<0.01$) and heifers ($P<0.02$). First service conception rates (FS-CR) were lower in cows positive for PUP ($P<0.01$). Moreover, presence of PUP and OUP affected FS-CR in heifers ($P<0.01$). Although A-CMD significantly affect FS-CR in cows ($P<0.04$), it did not affect FS-CR in heifers. Differences in average open day (OD) for cows and first service age (FSA) for heifers were significant between N-CMD ($P<0.02$) and A-CMD ($P<0.01$) groups, respectively. Our findings indicated that pathogens have a negative effect on reproductive performance of cows and heifers when it changes to appearance of CMD.

Key Words: Cervical Mucous Discharge, Reproductive Performance, Bacteria-Fungi

W207 *In vitro* production of bovine embryos in chemically defined serum-free media. A. Dhali, V. M. Anchamparuthy, S. P. Butler, R. E. Pearson, and F. C. Gwazdauskas*, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

Embryo production and culture in serum supplemented media is a common practice. Nevertheless, several studies indicate the need to develop suitable serum-free conditions for *in vitro* embryo production and culture as serum can inhibit embryo development and can alter morphological and chemical properties of embryos. Moreover, *in vitro* embryos generated under chemically defined conditions can serve as a valuable model in many research applications. The objective was to develop a complete chemically defined serum-free system for in vitro production and culture of bovine embryos. Abattoir-derived oocytes were matured in TCM-199 supplemented with LH (0.02 U/mL), FSH (0.02 U/mL), estradiol (1 µg/mL) and BSA. The swim-up separation of motile spermatozoa for *in vitro* fertilization (IVF) was performed in BSA supplemented HEPES buffered synthetic oviductal fluid (SOF). Matured oocytes were fertilized in BSA supplemented SOF-IVF medium and presumptive zygotes were cultured for 8 d (in a humidified 5% CO_2 atmosphere at 38.5°C) in BSA supplemented SOF-in vitro culture (IVC) medium containing either epidermal growth factor (EGF, 10 ng/mL), stem cell factor (SCF, 50 ng/mL), or IGF-1 (100 ng/mL). Control embryos were cultured in BSA and fetal calf serum (FCS) supplemented SOF-IVC medium. The cleavage rate did not vary significantly among the treatments and control (EGF: 77.6%, SCF: 68.3%, IGF-1: 73.4% and FCS: 72.8%). Similarly, the blastocyst (EGF: 22.7%, SCF: 24.6%, IGF-1: 26.0%, FCS: 35.2%) and expanded blastocyst (EGF: 10.6%, SCF: 12.7%, IGF-1: 16.5% and FCS: 22.7%) formation rates based on total number of oocytes did not differ significantly among the treatments and control. In conclusion, an acceptable level of embryonic development could be achieved using the serum-free chemically defined conditions and growth factor supplementation in the culture medium.

Key Words: Embryo, Bovine, Serum-free Media

W208 Droplet vitrification method did not induce cytoskeletal damage in mouse embryos. A. Dhali, V. M. Anchamparuthy, S. P. Butler, R. E. Pearson, and F. C. Gwazdauskas*, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

The concept of ultra rapid vitrification has been emerging in recent years. This particular process increases cooling and warming rates that provide the opportunity to reduce cryoprotectant concentrations in vitrification solutions and reduce cytotoxicity. The objective was to document embryo survival and cytoskeletal damage when mouse embryos were vitrified using a modified ultra rapid vitrification method, the droplet vitrification. Mouse embryos (zygotes, 2-cell and morulae) were initially equilibrated for 3 min in 50% vitrification solution and then quickly washed 3 times in vitrification solution (17.5% ethylene glycol (EG), 17.5% DMSO, 0.5M sucrose and 4 mg/mL BSA in M2 medium). Subsequently a drop (5µL) of vitrification solution containing 10 to 12 embryos was formed, placed directly onto liquid nitrogen and liquid nitrogen was poured immediately over the drop. Warming and removal of cryoprectants were performed by placing the vitrified drop into dilution medium (0.3M sucrose and 4 mg/mL BSA in M2 medium) for 3 min and then into M2 medium for 5 min. Following vitrification, warming and culture, 57.9% of pronuclear stage, 59.2% of 2-cell stage and 86.3% of morulae developed into blastocysts. The corresponding figures for hatched blastocyst were 37.8, 40.7 and 73.5%, respectively. Still, the development of control embryos into blastocyst (70.2% of pronuclear stage, 75.5% of 2-cell stage embryos and 91.5% of morulae) and hatched blastocyst (58.5% of pronuclear stage, 62.9% of 2-cell stage and 77.7% of morulae) was significantly ($P < 0.05$) higher. Laser scanning confocal microscopy revealed no actin cytoskeletal damage in any of the 3 stages of mouse embryos either after vitrification and warming or following the development into blastocyst. The study reveals that droplet vitrification is an easy and potentially ultra rapid vitrification method, which may be utilized to preserve oocytes and embryos of other species as well.

Key Words: Embryo, Cytoskeleton, Vitrification

W209 Association of oviductal fluid (ODF) proteins with the bovine zona pellucida. E. Monaco*[1], B. Gasparrini[2], L. Boccia[2], A. De Rosa[2], L. Attanasio[2], G. Campanile[2], and G. Killian[1], [1]*The Pennsylvania State University*, *State College*, [2]*Federico II University*, *Naples, Italy*.

The objective of this study was to determine using confocal microscopy whether bovine serum albumin (BSA), osteopontin (OPN) and lipocalin-type prostaglandin D synthase (L-PGDS) were associated with the bovine zona pellucida (ZP) of immature bovine oocytes and after in vitro matured oocytes were incubated in ODF. Ampullary (A) and isthmic (I) non luteal (NL) ODFs from two Holstein cows were used. To investigate if BSA, OPN, L-PGDS bind to the ZP alone or to cumulus cells and plasma membrane, mature cumulus oocyte complexes, denuded and ZP-free mature oocytes were incubated in 1) TALP medium (without BSA, with 0.1% PVA) (control), 2) TALP medium and 50% ANL-ODF, 3) TALP medium and 50% INL-ODF. After 2.5 h the eggs were washed and incubated for 1 h with primary antibodies against one of the three proteins, washed again and then incubated for 30 min with goat anti-rabbit FITC labeled secondary antibody. Immature denuded oocytes were incubated for 1 h in 1) TCM (control), 2) TCM and primary antibody to one of the three proteins and then for 30 min with FITC-labeled secondary antibody. In all cases, the ZP of mature denuded eggs probed with primary and secondary FITC labeled antibodies fluoresced when eggs were pre-exposed to either TALP medium alone or TALP medium and ANL/INL-ODF. In no case did the cumulus cells or the plasma membrane have an affinity for any of the three proteins. However, L-PGDS distribution differed from that of BSA and OPN in that it was detected in the perivitelline space. These studies suggest the presence and in vivo binding of these proteins to the eggs before they reach the oviduct. Because the ZP of immature denuded oocytes incubated in TCM and probed with primary and secondary FITC labeled antibodies also fluoresced like the mature eggs, we suggest that BSA, OPN and L-PGDS are first acquired by the ZP from follicular fluid during follicular development. (USDA grant 2004-34437-15106).

W210 Decreased pulsatile LH secretion does not affect the function of the corpus luteum of pregnancy in cattle. H. T. Toriz*, H. Basurto, A. A. Porras, and C. G. Gutierrez, *Facultad de Medicina Veterinaria. UNAM*, *Mexico DF, Mexico*.

The objective of this study was to determine the corpus luteum (CL) dependence of pulsatile LH secretion in pregnant cattle. Twelve cows

with 90 days of pregnancy were either used as controls (n=6) or treated chronically with a GnRH agonist (n=6) (Buserelin; Hoechst Marion Roussel Ltd.). The chronic treatment with GnRHa was given with Buserelin acetate released continuously at 2.5 µg/h by an Alzet osmotic. This treatment has been previously shown to block LH pulses (Gong et al, 1996). The pump was replaced every 28 days with a new pump throughout gestation. Cattle were bled twice weekly from day 90 of to day 150 of gestation and every fortnight thereafter until parturition. The ability of the pituitary from the control and GnRHa treated cows to release LH was tested forty days after the start of the study (aprox. day 130 of gestation) by injecting them with 50ug of buserelin. Progesterone and LH were measured by RIA. All cows continue their pregnancy and calved normally. After the first GnRHa pump was inserted, treated cows developed a secondary CL. Progesterone concentrations were higher ($p<0.05$) in the GnRHa treated cattle (12.8 ng/ml) than in the control group (8.8 ng/ml) throughout the remaining of pregnancy. In the control group, all cows responded with LH release (≥ 4 ng/ml) after the buserelin challenge. However, LH released was significantly reduced ($p<0.01$) by GnRHa chronic treatment. The results of this study suggest that the corpus luteum of pregnancy does not depend on pulsatile release of LH for its maintenance and function.

Key Words: Corpus Luteum, Luteinizing Hormone, Pregnancy

W211 Protective effects of the antioxidant dithiothreitol (DTT) on preimplantation bovine embryos exposed to heat shock. L. A. de Castro e Paula* and P. J. Hansen, *University of Florida*, *Gainesville*.

Effects of heat shock (HS) on bovine embryos are greater in culture under high oxygen (20.95%) when compared to low oxygen (5%). It was hypothesized that HS effects involve reactive oxygen species (ROS) and DTT reduces these effects. For Experiment (Exp) 1 (culture in high oxygen), two-cell embryos were cultured at 38.5°C (control) or 41°C (HS) for 15 h with 0, 50 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in the same DTT treatment (trt) and then at 38.5°C without DTT until day 8. DTT increased the percent of control embryos becoming blastocysts ($P<0.05$) and heat shock reduced blastocyst development ($P<0.05$). This reduction was less for embryos treated with 500 µM DTT ($P<0.05$). For Exp 2, two-cell embryos were cultured at 38.5°C or 41°C for 15 h in high or low oxygen and with 0 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in either high or low oxygen in the same DTT trt and then cultured in low oxygen at 38.5°C without DTT until day 8. HS decreased blastocyst development in all trts except for the 0 µM DTT group cultured in low oxygen (temp × DTT; $P<0.05$). For Exp 3 (culture in high oxygen), embryos ≥16 cells were cultured at 38.5°C or 41°C for 15 h in the presence of 0, 50 or 500 µM DTT. Embryos were then cultured at 38.5°C for 9 h in their same DTT trts and then at 38.5°C without DTT until day 8. DTT increased the percent of control embryos becoming blastocysts ($P<0.05$). HS reduced blastocyst development ($P<0.05$) but the reduction was less for embryos treated with DTT ($P<0.05$). Exp 4 was conducted as for Exp 3 except that embryos were fixed 24 h after start of HS and analyzed by TUNEL assay. The percent of TUNEL-positive cells was increased by HS in the absence of DTT ($P<0.05$) but not in the presence of 50 or 500 µM DTT ($P>0.1$). In summary, DTT improved development of embryos cultured in high oxygen and conferred partial protection from HS. Protection was incomplete and it is likely that there are ROS-independent actions of HS. Since DTT was detrimental to HS embryos in low oxygen, there may be a ROS-dependent thermoprotective mechanism deployed by the embryo in low oxygen.

Key Words: Embryo, Heat Shock, Antioxidant

W212 Nylon mesh vitrification for cryopreservation of bovine oocytes. V. M. Anchamparuthy*, A. Dhali, S. P. Butler, R. E. Pearson, and F. C. Gwazdauskas, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

Cryopreservation of oocytes is a challenge. The objective was to vitrify large numbers of cumulus intact bovine oocytes obtained from follicles of different diameter, ≤ 4 mm (Small) and 4 to 10 mm (Medium), after 15 h of in vitro maturation. Vitrification-warmed oocytes were in vitro fertilized using frozen semen from 2 bulls (Bull I and II). After maturation oocytes were first immersed in cryoprotectants consisting of 10% (v/v) ethylene glycol (EG), 4.5% (w/v) Ficoll-70 (F-70) and 0.075 M sucrose in Ca++ free PBS for 7 min followed by immersion in a solution consisting of 20% (v/v) EG, 9.0 % (w/v) F-70 and 0.15 M sucrose for 2 min, and finally in a solution of 40% (v/v) EG, 18% (w/v) F-70 and 0.3 M sucrose for 1 min. After equilibration, 15 to 20 oocytes were loaded onto nylon mesh, transferred to 2 mL pre-cooled cryovials, and directly plunging into liquid nitrogen. Thawing was conducted with a sequential series of 0.5, 0.25 and 0.125 M sucrose dilutions for 1, 2, and 3 min, respectively. After thawing, the oocytes were placed into maturation medium for an additional 9 h. Thawing resulted in 97% morphological survival with intact cumulus cells in both populations of oocytes. There was a difference ($P < 0.05$) in development rates between vitrified and control groups (39.6 ± 0.02 and 59.8 ± 0.02%, respectively, for cleavage compared with 6.5 ± 0.01 and 21.2 ± 0.01%, respectively, for blastocysts). Cleavage and blastocyst rates in oocyte populations from Small and Medium follicles were different ($P < 0.05$; 45.4 ± 0.02 and 53.9 ± 0.02% for cleavage; 11.6 ± 0.01 and 16.1 ± 0.01% for blastocyst, respectively). Sire did not affect cleavage rate ($P > 0.05$; 49.6 ± 0.02), but had an effect ($P < 0.05$) on blastocyst rate (10.0 ± 0.01 for Bull I vs. 17.8 ± 0.01% for Bull II). Our results show that nylon mesh is a useful method for vitrification of large numbers of matured bovine oocytes.

Key Words: Bovine, Vitrification, Oocyte

W213 Follicle numbers on the ovaries of cows selected for high and low IGF. L. Snellgrove[1], T. A. Hoagland*[1], G. W. Kazmer[1], M. E. Davis[2], D. Schrieber[1], and S. A. Zinn[1], [1]*University of Connecticut*, *Storrs*, [2]*The Ohio State University*, *Columbus*.

Post-partum lactating Angus beef cows (n = 10) divergently selected for greater (H; n = 6) or lesser (L; n = 4) IGF-I concentrations were subjected to rectal palpation and ultra-sonography to determine the influence of this divergent selection for IGF on follicular dynamics. Beef cows treated with bST have greater numbers of small and medium follicles. With these genetically diverse cows, the role of IGF on follicular development could be investigated. The cows were subjected to rectal examinations twice per week between 30 and 80 d post-partum. The number of follicles on each ovary were determined and classified as small (1 to 3mm), medium (4 to 6mm) or large (< 6mm). In addition, presence of corpora lutea, and length and width

of the ovaries were recorded. Total number of small, medium and large follicles were greater (P = 0.0068) in H IGF cows compared with L IGF cows. The H IGF group averaged 10.9 ± 0.76 follicles compared with the L IGF group which averaged 6.56 ± 0.93 follicles. On the right ovary, the number of small (3.87 ± 0.45 vs. 2.12 ± 0.55; P = 0.0389) and medium (1.28 ± 0.15 vs. 0.66 ± 0.18; P = 0.0284) follicles were greater in H IGF cows than L IGF cows. Similarly, on the left ovary, the number of medium follicles was greater (P = 0.046) in the H cattle (1.63 ± 0.21) compared with L cattle (0.84 ± 0.26); and the number of small follicles were greater at the P = 0.12 level in H (2.9 ± 0.35) then L (2.0 ± 0.42) cows. The average ovarian volume (4.8 ± 1.0 cc) or the number of large follicles (0.64 ± 0.1) on either the right or left ovary in the H or L IGF selected cows were not different (P < 0.12). In conclusion, cows selected for greater IGF had more follicles than cows selected for low IGF indicating that divergent selection for IGF subsequently plays a role in follicular recruitment.

Key Words: IGF, Beef Cows, Follicles

W214 Effect of insulin-like growth factor-1 during culture on blastocyst mRNA abundance and survival in utero to day 14 of bovine embryos produced in vitro. J. Block*[1], C. Wrenzycki[2], D. Herrman[2], T. M. Rodina[1], H. Niemann[2], A. D. Ealy[1], A. E. Fischer-Brown[3], and P. J. Hansen[1], [1]*University of Florida*, *Gainesville*, [2]*Institute for Animal Science*, *Neustadt, Germany*, [3]*University of Illinois*, *Urbana*.

Transfer of bovine embryos cultured with insulin-like growth factor–1 (IGF–1) can increase pregnancy rates in heat-stressed, lactating dairy cows. Two experiments were conducted to determine the effect of IGF–1 on the relative abundance of several developmentally important genes as well embryo survival to day 14 of gestation. In experiment 1, embryos were produced in vitro (n = 4 replicates) and cultured with or without 100 ng/mL IGF-1 for seven days. On day 7, grade 1 expanded blastocysts (n = 104 control and 96 IGF–1, respectively) were selected for semi-quantitative reverse transcription-polymerase chain reaction analysis. Treatment with IGF–1 increased (P < 0.02) the relative abundance of IGF binding protein 3 and desmocollin II and tended (P < 0.08) to increase sodium/potassium ATPase and Bax. In contrast, IGF–1 decreased (P < 0.05) the relative abundance of heat shock protein–70 and tended (P < 0.08) to decrease IGF-1 receptor. In experiment 2, non-lactating (n = 52) and lactating (n = 32) Holstein cows were selected as recipients following synchronization for timed-embryo transfer (n = 11 replicates). Embryos were produced as described in experiment 1. At Day 7 after anticipated ovulation (Day 0), a single embryo was randomly transferred to each recipient. Embryos were recovered at Day 14 and embryo length was recorded. Recovered embryos were cultured for 24 h and interferon-τ (IFN-τ) secretion was assessed using an anti-viral assay. Recovery rate at Day 14 for recipients that received IGF–1 treated embryos tended (P = 0.10) to be greater (16/37 = 43.2%) than for recipients that received control embryos (12/47 = 25.5%). However, there was no effect of IGF-1 on embryo length or IFN-τ secretion. Results indicate that IGF–1 treatment can alter the relative abundance of several developmentally important genes. Moreover, IGF–1 can increase embryo survival as early as day 14 of gestation. These effects may be important for the improved survival of IGF–1 treated embryos reported previously.

Key Words: Insulin-Like Growth Factor-1, Embryo, Bovine

W215 Effect of supplementation with Megalac-E on pregnancy rate in primiparous Nellore cows. C. N. Lopes[1], J. L. M. Vasconcelos*[1], T. P. B. Araujo[2], and L. O. F. Oliveira[3], [1]*FMVZ-UNESP*, *Botucatu, SP, Brazil*, [2]*Arm&Hammer*, *Brazil*, [3]*Propec Consultoria*, *Brazil*.

Fat supplementation has often positively influenced the reproductive status of the dairy cow. Linoleic acid is inhibitor of cyclooxygenase in endometrial tissue of dairy cows, which suppress endometrial secretion of PGF2α and potentially prevent early embryonic death (Staples et al, 1998). The objective was to evaluate effect of supplementation with Megalac-E® (40% linoleic acid; Arm&Hammer) on reproductive responses in primiparous Nellore cows after timed AI. The trial was conducted at a beef farm in Brazil from December 2006 to January 2007. Primiparous Nellore cows (n=411; 50 to 80 days post partum) received 0.4kg/day of concentrate plus minerals at pasture, from the beginning of the synchronization protocol until day 28 after timed AI and were randomly assigned to received or not the supplementation with Megalac-E: Control Group 100gr Kaolin (n= 211); Megalac Group 100 g Megalac-E (n= 200). Cows were synchronized with an intravaginal P4 device (CIDR®, Pfizer, Brazil) plus an injection of 2 mg of estradiol benzoate (Estrogin®, Farmavet, Brazil) on day 0. On day 9, 12.5 mg of dinoprost (Lutalyse®, Pfizer, Brazil) plus 0.5 mg of estradiol cipionate (ECP®, Pfizer, Brazil) were administered, CIDR® was removed, and the calves were removed until finishing TAI, that was performed 48 hours after CIDR® removal. Pregnancy rates were the percentage of cows diagnosed pregnant by ultrasonography (Aloka 500, probe 7.5 MHz) at Day 28 after TAI. Effects of treatment on pregnancy rates were analyzed by chi-square test. Pregnancy rates in the cows treated with Megalac-E (56.5%; 113/200) were higher (p=0.015) than in control group (45.6%; 94/211. The mechanism by which Megalac improves reproductive performance may be due to the Linoleic acid that has been demonstrated had inhibitory effect on the prostaglandin synthesis. This data suggests that Megalac-E at the beginning of the Timed AI protocol until day 28 after AI to lactating primiparous Nellore cows increased pregnancy rate, probably due its antiluteolytic effect.

Key Words: Conception, Megalac, Nellore Cows

W216 Progesterone postpartum determination and reproductive performance of crossbred cows. M. S. Arellano-Cornejo[1], J. C. Martinez-Gonzalez*[2], E. M. Romero-Trevino[1], F. Briones-Encinia[2], F. De la Garza-Requena[2], and M. Dominguez-Munoz[3], [1]*Instituto Tecnologico Superior de Altamira*, *Altamira, Tamaulipas, Mexico*, [2]*Agronomia y Ciencias, Universidad Autonoma de Tamaulipas*, *Ciudad Victoria, Tamaulipas, Mexico*, [3]*FMVZ, Universidad Autonoma de Tamaulipas*, *Ciudad Victoria, Tamaulipas, Mexico*.

This study was conducted to determine the effect of calving number (CN), body condition (BC), and days after calving (AC) on first progesterone postpartum rise (P4). Also first estrous postpartum (EP) and calving conception intervals (CC) were studied. Twenty one cows Brown Swiss × Zebu were divided into 4 groups according to CN: I) 3 (first-lactating); II) 9 (two calving); III) 7 (three calving), and IV) 2 (four calving). Cows grazed Cynodon nlemfuencis and Panicum maximum, and were protein supplemented (18% CP) with 2 kg/cow/d. Estrus was detected by daily visual observation at milking time and on pasture. Progesterone was determined by RIA, low plasma progesterone values (<0.5ng/mL) were consistent with ovarian inactivity, confirming

the true anestrus status of experimental animals. CC was confirmed by rectal palpation. BC was evaluated using a scale of 1 to 9, were 1 = very thin, and 9 = very fat. The study has duration of 60 days postpartum. The mean (P4P) was 1.07 ± 1.68 ng and were affected by BC and AC ($P < 0.001$). Cows with BC 3 and 4 failed to exhibit estrus and maintained low progesterone concentrations throughout the study (2.86, 1.89, 1.26, 0.61 and 0.04 ng for 7, 6, 5, 4 and 3 BC, respectively). The animals, high progesterone values from day 45 onwards suggested ovulatory estrus. It was concluded that BC and AC are the parameters that have more influence on ovarian activity ailed the duration of the anestrous postpartum period. To determine the factors that affect interval calving, it is necessary to study the overall herd fertility.

Key Words: Bovine, Progesterone, Anestrus

W217 Diagnosis of bovine freemartinism by fluorescence in situ hybridization using a bovine Y chromosome-specific DNA probe. S. H. Sohn, E. J. Cho, W. J. Son, and C. Y. Lee*, *Jinju National University*, *Jinju, Korea*.

A heifer born as a co-twin to a bull mostly becomes a sterile freemartin which needs to be screened out from the replacement stock during early development for efficient cattle production. Various methods are available for the diagnosis of freemartinism, but none of them are perfect in terms of the speed, sensitivity, or specificity. The present study was thus conducted to develop and validate a satisfactory fluorescence in situ hybridization (FISH) procedure for identifying the bovine XX/XY-karyotypic chimerism, the hallmark of the freemartinism. A FISH probe containing the 54-bp bovine male-specific BC1.2 DNA sequence was synthesized and labeled with digoxigenin by polymerase chain reaction. The FISH was performed on chromosome spreads and nuclei of blood lymphocytes on slides; karyotyping was done on the chromosome spread following G-banding. Upon FISH, the probe expectedly bound to the nucleus of the male cell or to a region of the Yp12 locus on the chromosome spread. Out of a total of 24 Holstein-Friesian and Korean Cattle heterosexual twins consisting of 13 heifers and 11 bulls which were analyzed in the present study, all but three exhibited the XX/XY-karyotypic chimerism to varying extents regardless of the breed or age of the animal in both FISH and karyotyping. One heifer was identified to have 100% XX-type cells by both analyses, whereas two bulls were judged as 100% XY- and XX/XY-chimeric karyotypes by karyotyping and FISH, respectively. Nevertheless, the ratios of the XY to XX cells in these two bulls and all the other animals were very similar between the two analyses. Results indicate that the present FISH is a rapid and reliable procedure which can be used for early diagnosis of bovine freemartinism.

Key Words: Freemartin, Karyotype, FISH

W218 Influence of insulin on plasma and hepatic composition, ovarian activity and estrous behavior in early lactation dairy cows. J. A. Casas*, M. F. Sa Filho, C. Narciso, F. Rivera, and J. E. P. Santos, *Veterinary Medicine Teaching and Research Center, University of California Davis*, *Tulare*.

Objectives were to determine the effects of increased plasma insulin under normoglycemia on blood and hepatic composition, ovarian activity and estrous behavior in dairy cows. Holstein cows at 1 d in milk (DIM) were randomly assigned to receive 0 (CON, n=38) or 75 IU of slow-release insulin (INS, n=38) and 30 g of glucose i.v. daily until 14 DIM. Ovaries were examined by ultrasonography 4 × weekly from 10 to 60 DIM. Estrous behavior was evaluated by a radiotelemetric heat mount device. Blood was sampled daily immediately before insulin injection in the first 14 DIM, and 4 × weekly thereafter. Plasma was analyzed for concentrations of glucose, insulin, nonesterified fatty acids, 3-hidroxybutyrate, insulin-like growth factor-I, growth hormone, estradiol and progesterone. Concentrations of glucose and insulin were also evaluated every 3 h during a 24 h after treatment. A liver biopsy was collected at 7 and 14 DIM and assayed for mRNA for bovine GH receptor and chemical composition. Yields of milk and components were measured for the first 90 DIM, and body condition (BCS) was scored at 1 and 60 DIM. Data were analyzed using the MIXED and Chi-square procedures of SAS (2001). In the first 24 h after treatment, concentrations of glucose in plasma were similar ($P=0.15$) and averaged 57.2 ±1.8 and 53.4 ±1.7 mg/dL for CON and INS cows. Rectal temperature was similar ($P=0.64$) for CON and INS during the first 10 DIM and averaged 39.3 oC. For CON and INS, respectively, yields (kg/d) of milk (40.7 vs 39.1), 3.5% fat-corrected milk (41.8 vs 41.5), energy corrected milk (37.8 vs 37.3), milk fat (1.50 vs 1.52) and true protein (1.17 vs 1.12) were similar ($P>0.10$). Similarly, mean and median BCS did not differ ($P>0.10$) for CON (2.75 and 2.84) and INS (2.63 and 2.71). All cows ovulated and DIM at first ovulation was similar ($P=0.80$) and averaged 20.5 d. Double ovulation was similar ($P=0.82$) between treatments and averaged 39.3%. Length of the first luteal phase did not differ between treatments and averaged 23.4 d. Treatment with insulin did not affect lactation performance or reproductive parameters evaluated.

Key Words: Dairy Cow, Follicle, Insulin

W219 Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. T. Tanaka*, M. Arai, S. Ohtani, S. Uemura, S. Kim, T. Kuroiwa, and H. Kamomae, *Tokyo University of Agriculture and Technology*, *Fuchu, Tokyo, Japan*.

The aim of the present study was to determine ovarian changes preceding the resumption of the ovarian cycle and their association with body energy status in postpartum dairy cows with different parities. In postpartum primi- (n=6), bi- (n=4) and multiparous (n=6) Holstein dairy cows, ovarian ultrasonographic observation starting at seven days after calving was performed every other day as a rule but daily after the confirmation of clinical signs of estrus for the detection of postpartum first ovulation. Blood samples were collected at ultrasonography and analyzed for estradiol and progesterone to monitor ovarian activity. To evaluate the nutritional condition of cows, body weight and body condition score (BCS, 1 = emaciated and 5 = obese) were measured weekly and blood samples for the analysis of glucose, insulin and non-esterified fatty acid (NEFA) were collected at the same time until postpartum second ovulation. The days to first ovulation after calving and the number of follicular waves preceding the first ovulation in primiparous cows were significantly greater than those in multiparous cows (31.8 ± 8.3 vs. 17.3 ± 6.3 days and 2.7 ± 0.8 vs. 1.3 ± 0.8 waves, $p<0.05$), but were not significantly different from biparous cows (28.8 ± 8.6 days and 2.0 ± 0.7 waves). Estradiol concentration on the day prior to the first ovulation in primiparous cows was significantly lower than that in multiparous cows (4.8 ± 2.3 vs. 9.6 ± 4.4 pg/ml, $p<0.05$). BCS was maintained at a

level of more than 2.5 during the postpartum period in all cows and the influence of parity on postpartum changes in BCS, glucose, insulin and NEFA was not found throughout the experiment. The present study demonstrated that the interval from calving to first ovulation in primiparous cows was longer than that in multiparous cows in association with the number of follicular waves under such similar body nutritional conditions.

Key Words: Parity, Postpartum First Ovulation, Dairy Cows

W220 Pregnancy loss in lactating Holstein cows diagnosed with twin versus singleton fetuses. N. Silva del Río*[1], J. D. Colloton[2], and P. M Fricke[1], [1]*University of Wisconsin*, *Madison*, [2]*Bovine Services LLC*, *Edgar, WI*.

Our objective was to characterize pregnancy loss (PL) for cows diagnosed with twin (T) vs. singleton (S) fetuses on a commercial dairy farm comprising 1,100 lactating Holstein cows. Records were collected by the herd veterinarian who performed weekly reproductive examinations using transrectal ultrasonography from January 2005 to February 2006. The initial data set included 2,048 pregnancy examinations (1,389 initial examinations and 659 re-examinations). The 730 observations with no recorded re-evaluation included 673 cows diagnosed not pregnant, 22 cows with nonviable fetuses, 33 cows diagnosed pregnant with a singleton fetus, and 2 cows diagnosed pregnant with twin fetuses. The remaining cows were either culled from the herd or were re-inseminated. Only records from cows diagnosed pregnant with a viable fetus from 27 to 40 d postbreeding that included a pregnancy re-evaluation from 48 to 82 d postbreeding were used to assess PL. Cows (n=13) identified with twin fetuses at the pregnancy re-examination but identified with singleton fetuses at the initial pregnancy examination were excluded from the data set. A total of 468 S cows and 74 T cows were included in the final data set. Pregnancies of S cows with 1 CL comprised 60.9% right horn with a CL on the right ovary and 39.1% left horn with a CL on the left ovary. Pregnancies of T cows with 2 CL comprised 26.1% unilateral right horn with 2 CL on the right ovary, 31.9% unilateral left horn with 2 CL on the left ovary, and 42.0% bilateral horn with one CL on each ovary. Overall, PL was greater ($P<0.01$) for T (25%) vs. S (4.9%) cows. For S cows with 1 CL, PL was 5.5 % (22/379), whereas PL for S cows with 2 CL was 1.5% (1/66). Among T cows, only 5 had 1 CL, and 3 of these cows lost 1 fetus and 1 lost both fetuses. For T cows with 2 CL, 8.6% (6/69) lost 1 fetus and 13.0% (9/69) lost both fetuses. We conclude that PL is greater for T compared to S cows and that spontaneous loss of one fetus and maintenance of the other can and does occur for T cows.

Key Words: Twinning, Pregnancy Loss, Dairy Cow

W221 Effects of twin pregnancy and prepartum diet on early postpartum ovarian activity in Holstein dairy cows. N. Silva del Río*, R. R. Grummer, and P. M. Fricke, *University of Wisconsin*, *Madison*.

To evaluate the effect of pregnancy type [singleton (S) vs. twin (T)], and prepartum feeding management [transition diet (NEL=1.54 Mcal/kg) for 3 (3TR) vs. 8 wk (8TR) before expected calving date (ECD)], on postpartum (PP) ovarian activity and periparturient traits, multiparous (n=39) and primiparous (n=8) Holstein cows were used in a 2×2 factorial randomized complete block design. All cows were feed a late lactation diet (NEL=1.58 Mcal/kg) from 90 to 60 d before ECD and the same early lactation diet (NEL=1.71Mcal/kg) after calving. At dry-off, cows were feed a transition diet for 8 wk before ECD (8TR) or far-off diet (NEL=1.32 Mcal/kg) for 5 wk followed by transition diet 21d before ECD (3TR). Thrice weekly (MWF), ovaries were evaluated with transrectal ultrasound, and blood samples were collected for serum progesterone (P4). Gestation length was shorter ($P<0.05$) for T than for S cows (276 vs. 281 d). Calf BW was greater ($P<0.05$) for twin vs. singleton calves (70.7 vs. 42.4 kg) and, for 5 of the twin pairs, the smaller calf was 24 % lighter than its cotwin. Numerically more retain placenta were recorded for T than for S cows (55 vs. 29 %), and more ($P<0.05$) T cows required calving assistance than S cows (70 vs. 30 %). Although prepartum serum P4 (-7 to 1 d relative to calving) was greater ($P<0.05$) for T than for S cows (2.5 vs. 1.9 ng/mL), no effect of treatment was detected for average d to first PP ovulation (40 ± 5 d); average d to first PP 10 mm follicle (15.0 ± 1.8 d); and diameter of the largest follicle at first PP ultrasound (7.0 ± 0.6 mm). No treatment effects were detected for the incidence of anovulation (9.1%) or short luteal phases (11.4 %). No treatments effects were detected on ovulation rate of the first PP dominant follicle (57.5%) or double ovulation rate at first (29.7%) or second PP ovulation (16.1 %). Thus, although T cows have a greater risk for negative periparturient traits than S cows, no effect of treatment was detected for PP ovarian activity.

Key Words: Twinning, Transition Diet, Early Postpartum Ovarian Activity

W222 Relationship between the occurrence of first ovulation in early postpartum and metabolic status in the cows that experiencing postpartum disease. M. Matsui*, E. Kaneko, M. Kataoka, C. Kawashima, N. Sudo, N. Matsunaga, M. Ishi, K. Kida, Y.-I. Miyake, and A. Miyamoto, *Obihiro University of Agriculture and Veterinary Medicine*, *Obihiro, Hokkaido, Japan*.

Our recent study showed that the cow with early first ovulation (1st OV) by 3 weeks postpartum (wk pp) has higher subsequent fertility, and that the metabolic status closely relates to the occurrence of early 1st OV. It is generally accepted that the cow with postpartum disease has delayed resumption of ovarian cycle and low fertility. In present study, the relationship between the occurrence of early 1st OV and metabolic status in cows experiencing postpartum disease was examined. We analyzed Holstein cows that had clinical record for postpartum paraplegia, retained placenta, abomasal displacement, mastitis and/or ketosis by 100 days pp. Weekly blood samples were obtained from 81 multiparous and 23 primiparous cows from 1st to 9th wk pp, and measured metabolites (glucose, non-esterified fatty acid: NEFA, aspartate aminotransferase: AST, γ-glutamyl transpeptidase: γ-GTP), metabolic hormones (growth hormone: GH, IGF-I, insulin) and progesterone (P4) concentrations. Increase in plasma P4 (≥ 1 ng/ml) by 3 wk pp was observed in 37 multiparous (45.7%) and 10 primiparous cows (43.5%), where those cows were confirmed as having early 1st OV. All data were evaluated by repeated measure analysis of variance. The average and values at each week by 3 wk pp were analyzed by Student's t-test. Multiparous cows with early 1st OV showed higher glucose at 3rd wk pp, lower AST at 2nd and 3rd wk pp, lower average of γ-GTP during 3 wk pp, lower GH at 3rd wk pp and higher average of insulin during 3 wk pp compared to the cows

having no ovulation. Lower NEFA at 1st wk pp, lower average of NEFA during 3 wk pp, and higher insulin at 3rd wk pp were observed in primiparous cows with early 1st OV. Data suggest that metabolic status in early pp influences the occurrence of early 1st OV in cows that experience postpartum disease. Hepatic function in multiparous cows and body fat mobilization in primiparous cows appear to relate to early 1st OV in pp. In addition, it may be common to these multiparous and primiparous diseased cows that insulin levels by 3 wk pp closely associate with early 1st OV.

Key Words: Dairy Cow, First Ovulation, Postpartum Disease

W223 Effects of dietary fats differing in proportion of unsaturated fatty acids on characteristics of preovulatory follicles in dairy cows. M. Katz*[1,2], A. Arieli[2], and U. Moallem[1], [1]*Agriculture Research Organization*, *Bet Dagan, Israel*, [2]*Faculty of Agriculture, Hebrew University*, *Rehovot, Israel*.

The objectives of this study were to examine the effects of dietary prilled fat containing low proportion of unsaturated fatty acids (LUFA) or calcium soap of fatty acids (FA) containing high proportion of unsaturated FA (HUFA), on characteristics of preovulatory follicles. Israeli-Holstein dry cows, 256 d pregnant, were divided into three treatments that continued until 100 d in milk: 1) Control - (n=14) were fed a dry cow diet and postpartum were fed a lactating cow diet; 2) LUFA - (n=13) were supplemented with 230 g/d per cow of prilled fat; 3) HUFA - (n=14) were supplemented with 215 g/d per cow of calcium soap of FA. At 40 d in milk the estrus cycle was synchronized. Fourteen d after behavioral estrus, cows were injected with $PGF_{2\alpha}$ and 48 h later > 7 mm follicles were aspirated (aspiration ranged 55 - 70 d in milk). Androstenedione (A_4), estradiol (E_2), progesterone (P_4), insulin and nonesterified FA concentrations in follicles were determined. Follicles with E_2/P_4 ratio higher than 1 were analyzed (n=39). Follicular A_4 concentrations were 69% higher in HUFA and 65% lower in LUFA than in control (181.3, 37.4 and 107.3 ng/ml, respectively, $P < 0.04$). Follicular E_2 concentrations were 40% higher in HUFA and 53% lower in LUFA than in control (1695.6, 572.5 and 1214.7 ng/ml, respectively, $P < 0.02$). Total A_4 and E_2 in follicles were higher in HUFA than in control with no differences between LUFA and control. No differences were observed in P_4 concentrations and content among groups. The E_2/P_4 ratio was 47% higher in HUFA and 41% lower in LUFA than in control (21.5, 8.7 and 14.7, respectively, $P < 0.05$). Nonesterified FA and insulin concentrations in follicles were not different among groups. In conclusion, the positive effect of dietary unsaturated FA on preovulatory follicle hormones may be beneficial to the oocyte, could improve estrus intensity and therefore contribute to fertility in dairy cows.

Key Words: Unsaturated Fatty Acid, Preovulatory Follicle

W224 Effects of endothelin-1 infused chronically adjacent the luteal-containing ovary or intrauterine in ewes on luteal function. C. W. Weems*, Y. S. Weems, D. Johnson, T. Uchima, E. Lennon, A. Raney, K. Goto, G. Bowers, J. Saldana, and J. Pang, *University of Hawaii*, *Honolulu*.

Endothelin-1 (ET-1) has been reported to mediate PGF2α-induced luteolysis (Hinckley and Milvae, Biol. Reprod. 64:1619, 2001). mRNA for ET-1 converting enzyme-1, pre-pro ET-1 and ET receptors increased in cow corpora lutea (CL) from D-1-10 postestrus, were similar on D-10 and D-17, were not increased by PGF2α on D-10, but were increased by PGF2α on D-17 when luteolysis was underway (Choudhary et al., Domest. Anim. Endocrinol. 27:63, 2004). PGE (PGE1 and PGE2) is antiluteolytic and luteotropic (C. Weems, Y. Weems, R. D. Randel. The Vet. J. 171:206, 2006). ET-1 increased PGE secretion by cow CL in vitro when estrus was not synchronized or when synchronized with PGF2α and did not affect CL PGF2α or progesterone (P4) secretion. This does not support ET-1 being luteolytic (Y. Weems et al.; Prostaglandins and Other Lipid Mediators 74:45, 2003). The objective of this experiment was to determine whether ET-1 infused every 6 h from 2400 h on D-10 to 1800 h on D-18 of the ovine estrous cycle into tissue of the ovarian vascular pedicle (IP-2μg) adjacent the CL ovary or intrauterine (IU-4 μg) was luteolytlc in ewes. Treatments were: Vehicle-IP (n=4); Vehicle-IU (n=4); ET-1-IP (n=5); or ET-1-IU (n=5). CL weights at 1800 h on day 18 were analyzed by a 2×2 Factorial Design for ANOVA. Jugular venous plasma at 0, 18, 42, 66, 90, 114, 138, 162, and 186 h was analyzed for P4 by RIA. P4 profiles were analyzed by a Split Plot Design for ANOVA for repeated measures. CL weights differed ($P \leq 0.05$) among treatment groups. CL weights (mg) at 1800 h on D-18 were: VEH-IP-247±38; VEH-IU-195±31; ET-1-IP-626±74; and ET-1-IU-542±69. CL weights were heavier ($P \leq 0.05$) in ET-1 IP or IU-treated ewes than Vehicle-IP or IU treatment groups and did not differ ($P \geq 0.05$) between ET-1 IP or IU groups. Profiles of P4 in jugular venous blood of Vehicle IP or IU were lower ($P \leq 0.05$) than in ewes treated with ET-1 IP or IU, which did not differ ($P \geq 0.05$) between ET-1 IP or IU treatment groups. In summary, ET-1 prevented the decrease in CL weights and the decline in P4 compared to controls. It is concluded that ET-1 may not be luteolytic, but antiluteolytic in ewes.

Key Words: Corpus Luteum, Endothelin-1, Progesterone

W225 Effect of extender on retention of viability and motility in hair sheep and goat semen stored at 4°C. J. L. Mook* and S. Wildeus, *Virginia State University*, *Petersburg*.

Liquid storage of chilled semen may provide an opportunity to expand the use of AI in small ruminants when used in conjunction with overnight shipping. This study evaluated the ability of various extenders to maintain motility and viability in hair sheep and goat semen. Ejaculates were collected via artificial vagina during the breeding season. For trial 1, two separate collections were used to generate pooled samples of 4 animals/species. Samples were extended in 2.9% sodium citrate, DPBS, DPBS with BSA, and TRIS with egg yolk (EY). Extended samples were initially kept at 25°C for 3 hand were then stored at 4°C for 24 h. Motility was assessed using a CASA system, and viability with eosin and Sybr14/PI staining at 0, 3, and 24 h. For trial 2, ejaculates from 6 animals/species were extended individually in TRIS, TRIS with BSA, TRIS with EY, non-fat milk, and a commercial 2-step extender (Continental Plastic Corp.). Extended samples were kept at 25°C for 6 h, and were then stored at 4°C until 48 h. Motility and viability were determined at 0, 3, 6, 24, and 48 h, and at 0, 12 and 48 h, respectively. For trial 1, total (Tmot) and progressive motility (Pmot) were influenced by an interaction of extender and storage time ($P<0.001$). Initial buck Tmot and Pmot, and ram Pmot were similar across all extenders. Ram samples in EY-containing extenders maintained higher motility and viability for the duration of storage ($P<0.05$). Buck samples demonstrated no change in viability

over time. In trial 2, initial Tmot and Pmot were higher ($P<0.05$) in TRIS with either EY or BSA over time. Ram samples extended in TRIS with EY had reduced ($P<0.05$) Tmot and Pmot initially compared to the other extenders, but maintained the highest motility over 48 h. No changes in viability were detected via eosin staining after 48 h, but Sybr14/PI staining indicated viability in rams was only maintained ($P<0.05$) in EY and milk. In both trials, effects on motility were usually notable within the first 3 h. These results show EY-based extenders maintained motility and viablity best, and should be evaluated further for long-term chilled storage of buck and ram semen.

Key Words: Semen, Hair Sheep, Goats

W226 Use of fecal progestin determinations to characterize the estrous cycle in captive female bontebok (*Damaliscus pygargus pygargus*). M. McGee*[1], A. Kouba[2], S. Bowers[1], R. Meek[2], B. L. Elliot[2], C. Horton[2], T. Hill[2], E. Piorkowski[2], and S. Willard[1], [1]*Mississippi State University*, *Mississippi State*, [2]*Memphis Zoo*, *Memphis, TN*.

The bontebok is an antelope that almost became extinct and is classified as "vulnerable". Information concerning their reproductive physiology is needed in support of propagation efforts. Our objective was to profile fecal progestins (PROG) of female bontebok to characterize the estrous cycle and reproductive season. Samples were collected from enclosures with positive identification of animal-fecal pairs. Fecal samples (n=1,132) were collected from n=6 female bontebok from July 2002 to Sept. 2005. Samples were stored at -20°C until extraction using 0.5 g of feces in 5 mL 80% Methanol with shaking for 12-14 h. Samples were centrifuged for 15 min and the supernatant analyzed for immunoractive fecal PROG in a progesterone RIA. Coefficients of variation (CV%) are reported as a statistical measure of variability among estrous cycles. Length of the estrous cycle was 20.7±1.4 d (range: 12-26 d; CV: 19.8%). For females that did not become pregnant, the number of estrous cycles exhibited in a season was 4.5±0.5 cycles (CV: 15.7%). Shifts in the seasonality of cycles were observed among individuals from year-to-year, attributed to environment (season) and/or previous reproductive status. As a representative example, a bontebok female (#L13871) gave birth in July 2002 and began cycling 137 d later from Dec. 4, 2002 to April 3, 2003 exhibiting n=5 estrous cycles (length: 22.4±1.5 d; CV: 14.6%) and did not become pregnant, shifting into anestrus. The next year #L13871 exhibited n=4 estrous cycles (length: 18.5±2.2 d; CV: 23.6%) that began and ended earlier than the previous year: Sept. 9, 2003 to Jan. 11, 2004. It is unclear whether lactational-induced anestrus resulted in a later reproductive season in 2002/2003 than 2003/2004, or whether a seasonal cue may have initiated the shift in cycles. In conclusion, this study has identified characteristics of the estrous cycle and reproductive season (possibly affected by previous reproductive status and/or environment) in captive bontebok. These data may assist in the reproductive management of this species to improve propagation efforts.

Key Words: Bontebok, Fecal Hormones, Estrous Cycle

W227 Cloning and characterization of chicken prostaglandin E receptor subtypes 2 and 4 (EP2 and EP4). A. H. Y. Kwok*, C. Y. Wang, Y. Wang, and F. C. Leung, *The University of Hong Kong*, *Hong Kong, HK-SAR, China*.

Prostaglandin E2 (PGE2) belongs to the eicosanoids and is an important chemical mediator regulating many vital physiological processes, including muscle relaxation, vascular homeostasis, gastrointestinal function and maintenance of pregnancy. Prostaglandin E receptor subtypes 2 and 4 (EP2 and EP4) are crucial mediators between its ligand, PGE2, and downstream intracellular cyclic AMP/protein kinase A (cAMP/PKA) signaling pathway. Both receptors were identified in mammals and zebrafish, but they have not been cloned in the avian species. In the present study, using reverse transcription-polymerase chain reaction (RT-PCR), the full-length cDNAs for chicken EP2 and EP4 receptors were cloned from adult chicken ovary and testis respectively. The full-length cDNA of EP2 gene encodes a precursor of 475 amino acids with a high degree of amino acid identity to that of mammals, including human (87%), mouse (86%), rat (84%), dog (85%), and cow (83%), and a low sequence identity to zebrafish (52%). Chicken EP4 is 356 amino acids in length and also shows high amino acid identity to that of mammals (human, 61%; mouse, 63%; rat, 61%; dog, 58%; cow, 59%) and a lower sequence identity to lower vertebrate (zebrafish, 41%). Using the pGL3-CRE-luciferase reporter system, we also demonstrated that PGE2 strongly induces luciferase activity of CHO/DF-1 cells expressing EP2 in a dose-dependent manner (EC50: <1 nM), confirming the functionality of the cloned EP2 receptor. The same experiment is also under way for EP4. The cloning and characterization of EP2 and EP4 receptors would help us to establish a molecular basis to elucidate the physiological roles of prostaglandin E2 in target tissues including their actions in chicken ovary.

Key Words: Chicken, PGE2, Prostaglandin E Receptors

W228 Pulmonary hemodynamic responses to intravenous prostaglandin E_2 in broiler chickens. S. Stebel and R. F. Wideman, Jr.*, *University of Arkansas*, *Fayetteville*.

Prostaglandin E_2 (PGE_2) affects pulmonary arterial pressure (PAP), pulmonary vascular resistance (PVR), and respiratory rate (RR) in mammals but no information previously was available regarding avian pulmonary responses to PGE_2. Two experiments were conducted in which 45 to 55 d old male broiler chickens were infused i.v. with PGE_2 at the lowest rate (30 µg/min for 4 min) that reliably reduced PAP by approximately 2 mm Hg during pilot studies. When compared with pre-infusion (Control) values in Experiment 1, PGE_2 reduced PAP from 19 ± 1 to 16 ± 1 mm Hg ($P<0.05$) and reduced mean systemic arterial pressure (MAP) from 111 ± 6 to 81 ± 5 mm Hg ($P<0.05$) but did not significantly reduce heart rate (HR) (Control: 338 ± 9 beats/min; PGE_2: 320 ± 12 beats/min; $P>0.05$). Infusing PGE_2 also reduced the respiratory rate (RR) from 57 ± 2 to 46 ± 4 breaths/min ($P<0.05$), and reduced the percentage saturation of hemoglobin with oxygen ($\%HbO_2$) from 85 ± 2 to 77 ± 3 % ($P<0.05$). The PAP, MAP, RR and $\%HbO_2$ all recovered to control levels after the PGE_2 infusion ceased. In Experiment 2 an ultrasonic flow probe was surgically implanted to measure cardiac output (CO). When compared with pre-infusion control values, PGE_2 reduced PAP from 25 ± 2 to 21 ± 1 mm Hg ($P<0.05$), reduced CO from 140 ± 6 to 111 ± 5 mL/kg BW × min ($P<0.05$), and reduced RR from 49 ± 4 to 35 ± 4 breaths/min ($P<0.05$). All of these variables recovered to control levels within 9 min after stopping the PGE_2 infusion. The reduction in CO was caused by a reduction in HR rather than stroke volume. The PVR, calculated as PAP/CO, was not altered by PGE_2 (Control: 0.180 ± 0.012 relative resistance units; PGE_2: 0.200 ± 0.017 relative resistance units; $P>0.05$). These results indicate that in broilers PGE_2 reduced PAP by reducing

CO rather than by acting as a pulmonary vasodilator to lower PVR. Reductions in CO, RR and blood oxygenation triggered by i.v. PGE_2 infusion previously have been reported for mammalian species in which specific PGE_2 receptors reside on parasympathetic neurons, suggesting parasympathetic inhibition of cardiac and respiratory function may play a role in the responses of broilers to PGE_2.

Key Words: Respiration, Hemodynamics, Cardiac Output

W229 Coordinate accumulation of the egg envelope glycoproteins during follicular development in Japanese quail (*Coturnix japonica*). T. Sasanami*[1], M. Ohtsuki[1,2], G. Hiyama[3], N. Kansaku[3], A. Tsukada[4], K. Tahara[4], T. Watanabe[4], T. Yoshimura[4], and M. Mori[1], [1]*Shizuoka University*, *Shizuoka, Japan*, [2]*Gifu University*, *Gifu, Japan*, [3]*Azabu University*, *Sagamihara, Japan*, [4]*Nagoya University*, *Nagoya, Japan*.

The extracellular matrix surrounding avian oocytes, termed as perivitelline membrane (PL), exhibit a three-dimensional network of coarse fiber between the granulosa cells and the oocyte. Our previous studies of Japanese quail have demonstrated that one of its components, ZPC, is synthesized in the ovarian granulosa cells. Another component, ZP1, which critically involves in triggering the sperm acrosome reaction in quail, is synthesized in the liver, and is transported to the surface of the oocyte in the follicles. We have previously isolated cDNAs encoding quail ZPC and ZP1, and now report the isolation of cDNA encoding quail ZPA. By RNase protection assay and *in situ* hybridazation with radio-labelled antisense probes to ZP mRNAs, we have demonstrated that ZPA transcripts are detected in the granulosa layer of small white follicles (SWF). Expression level of ZPA decreased progressively during follicular development (n=3, $P < 0.01$), and the highest expression was observed in SWF. On the other hand, ZPC transcripts increased as corresponding to follicular development (n=3, $P < 0.01$), but barely visible in SWF. Immunohistochemical analyses using specific antisera raised against ZP glycoproteins indicate that the immunoreactive materials recognized with anti-ZPA antiserum were detected in the apical side of the granulosa layers of SWF, and were also detected in the PL of small yellow follicles (SYF). However, the signal was not detected on the sections of SWF, which had been reacted with anti-ZPC as well as with anti-ZP1 antiserum. The intense staining with anti-ZPC antiserum was seen in the PL of SYF, whereas the weak signal was appeared when the section of 4th largest follicles was stained with anti-ZP1 antiserum. These results indicate that three PL glycoproteins are accumulated in a coordinate manner during the follicular development in quail, and the phenomena might contribute to the formation of the insoluble fiber of avian PL.

Key Words: Egg Envelope, Zona Pellucida, Japanese Quail

W230 Nicarbazin reduces egg production and fertility in White Pekin ducks via reducing ZP3 in the perivitelline membrane. V. P. Reinoso*, R. Katani, and G. F. Barbato, *The Pennsylvania State University*, *University Park*.

This study determined the dose-response relationship for the effect of nicarbazin in reducing egg production and/or hatchability in White Pekin ducks. Six dosages of nicarbazin were fed to female ducks with the range of doses being 0 ppm, 31.25 ppm, 62.5 ppm, 125 ppm, 250 ppm and 500 ppm for 14 days (n=12/dose group; N=72). Ducks were individually caged and artificially inseminated weekly with semen from untreated drakes. Control ducks had an average rate of lay of 93% with a mean fertility of 87%. Ducks receiving either 250 or 500 ppm nicarbazin had significantly reduced egg production within 2 days of treatment ($P<0.05$). The remaining groups had significantly reduced egg production by within 8 days of treatment ($P<0.05$). By the end of the treatment, all nicarbazin groups had significantly lower egg production than the control groups ($P<0.01$). Recovery of egg production after withdrawal of the nicarbazin treated diets occurred in reverse order of treatment dose, beginning 2 days after drug withdrawal, and completely recovering within 4 days. All nicarbazin groups had significantly lower fertility than the control group within 3 days ($P<0.05$). By seven days of nicarbazin treatment, the 500 ppm group had no fertile eggs (laying at 20%). The 125 and 250 ppm treatments eliminated fertility by day 27 (or 12 days post-treatment; $P<0.01$). Recovery of fertility followed the inverse of nicarbazin dose in the diet, preceding recovery of egg production by 2-3 days. Western blot analysis suggested a reduction in the presence of immunoreactive ZP3 (zona pellucida protein 3) in the perivitelline membrane of eggs laid by nicarbazin treated ducks. Furthermore, there were far fewer (<5%) sperm trapped in the perivitelline membrane of eggs from nicarbazin-treated ducks than in those from controls ($P<0.01$). The decreased fertility, western blots and dearth of trapped sperm in eggs of nicarbazin treated ducks implies a direct and negative effect of nicarbazin on ZP3, the putative sperm receptor, in the avian oocyte.

Key Words: Nicarbazin, ZP3, Ducks

W231 Isolation and culture of chicken oocytes. W. D. Berry*, S. S. Oates, L. M. Stevenson, and C. R. James, *Auburn University Poultry Science*, *Auburn, AL*.

A method of isolation and culture of chicken oocytes was needed to advance studies of oocyte development. The isolation of immature chicken oocytes was accomplished by enzymatic digestion of the chick ovary to disperse the immature oocytes. Ovaries were harvested from newly hatched chicks. The ovaries were collected into 4C calcium/magnesium free Hanks balanced salt solution (HBSS). The ovaries were washed 3 times in HBSS containing antibiotics/antimycotics, then finely minced in the HBSS. The minced tissue was then incubated with agitation for 60 minutes at 37C in a sterile enzymatic dispersal solution containing hyaluronidase, type 2 collagenase, and pronase. The dispersal solution containing the digested tissue was then filtered through a 100 micron filter and centrifuged at 25×g, at 4C, for 7 minutes. The cell pellet was then resuspended in minimal essential medium (MEM) and repelleted by centrifugation, followed by another resuspension step. The cell suspension was then cultured for 6 hours in MEM to remove contaminating fibroblasts by attachment. Alternatively, contaminating fibroblasts were removed by centrifuging the cell suspension through a Percoll gradient. Following fibroblast removal, the isolated oocytes were pelleted by centrifugation, resuspended in MEM and counted on a hemocytometer. Each chick ovary yielded 12,000 to 25,000 viable oocytes. The isolated and purified oocytes were cultured for up to one week in supplemented Dulbecco's MEM-Hams F-12 medium. Oocytes were also preserved at -20C in 95% ethanol. This work was supported by the Alabama Agricultural Experiment Station and the U.S. Poultry and Egg Association.

Key Words: Oocyte, Isolation, Chicken

W232 Gene expression of hen granulosa cell (GC) steroidogenic enzymes and gonadotropin receptors following a chronic heat stress (HS) episode. H. Taira*[1] and M. M. Beck[2], [1]*University of Nebraska*, *Lincoln*, [2]*Clemson University*, *Clemson, SC*.

This study was conducted to determine the effect of HS on gene expression of several key enzymes and hormone receptors in the steroidogenic pathway in GC of laying hens and to determine whether the hen responds like the rat. HS reduces synthesis of and circulating luteinizing hormone (LH), progesterone (P_4) and estrogen in hens, interfering with reproduction. In earlier studies, GC from hens subjected to HS synthesized less P_4 , even when stimulated by LH and 3β-hydroxysteroid dehydrogenase (3β-HSD) activity was reduced, while incubation with LH+FSH increased the activity. In this study, three strains of Hy-Line® laying hens (W36, W98 and CV20) were subjected to 35C (HS) for two wk or maintained in a thermoneutral (TN, 22C) environment. GC from the largest follicles were isolated and enzymatically dispersed. Aliquots of 100,000 viable cells were incubated with or without LH+FSH and the expression of mRNAs of steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage (P450scc), LH and FSH receptor (LH-R, FSH-R) and 3β-HSD were analyzed using real time RT-PCR normalized to 18s. Compared to TN, HS did not affect the expression of mRNA of P450scc, LH-R or FSH-R in any strain (P>0.1). Expression of StAR mRNA was reduced by HS in GC in W36 (P=0.0033) but not W98 or CV20 (P=0.6422, P=0.5275, respectively). Reduced 3β-HSD mRNA expression was observed only in CV20 (P=0.0589) and hormone incubation did not improve gene expression in any strain. This is somewhat inconsistent with earlier studies, in which HS reduced GC 3Î²-HSD activity and increased the number of apoptotic GC. The lack of a correlation between expression of 3β-HSD mRNA and enzyme expression under HS may suggest that HS interferes at translational but not the transcriptional level, that other factor(s) (e.g., increased PRL) maintain enzyme stability or receptor regulation, or that the effect of incubation times of hormones on different genes varies. A similar lack of correlation between mRNA and enzyme activity may be true for StAR and P450scc. Different methodologies made direct rat comparisons difficult.

Key Words: Steroidogenesis, mRNA

W233 Some observations on molting male Japanese quail. B. K. Biswas and K. L. Arora*, *Fort Valley State University*, *Fort Valley, GA*.

Molting is one of the important factors that cause significant changes in physiological and morphological characteristics in Japanese quail (*Coturnix japonica*). Our observations on **molting males** made during October 2006 and February 2007 are reported here. Each male (n=20; age 130-50 d) was housed in a separate cage (LD=16:8) with females. The birds were weighed regularly and blood was collected from the wing vein for determining PCV, glucose, and total plasma proteins. The cloacal gland was also measured. Some birds were euthanized to observe the size of testes. The birds started loosing feathers around the neck, then chest and back. The birds ceased crowing and lost some weight during molting. The testes were significantly reduced in size (4-5 vs 14+ mm) and weight (40-50 mg vs ~1.8 g). The cloacal gland was also significantly reduced from 14-19 mm to 6-8 mm. At the same time, the fertility in terms of fertile eggs laid by the cage-mate females was reduced to zero with the reduction of cloacal gland size. After molting the birds regained feathers starting around the neck, followed by chest and then extended backward toward the tail. The cloacal gland as well as testes also regained its normal size and weight. Fertility in male returned to 100% which have regained the size of cloacal gland to a minimum of 14 mm. The PCV of some male birds decreases (53.2% vs. 48.1%; p>0.05) and total plasma proteins increases (3.6 vs 4.2 mg/dl; p>0.05) during molting. Our observations indicate that the size of cloacal gland, testes and fertility in males are interrelated, and the cloacal gland size can serve as an indicator for male capability to fertilize eggs.

Key Words: Japanese Quail, Molting, Cloacal Gland

W234 Rooster semen cryopreservation: Effect of line and male age on sperm function. D. C. Bongalhardo*[1], J. Pelaez[1], J. E. Fulton[2], S. Saxena[2], P. Settar[2], N. P. O'Sullivan[2], J. Arango[2], and J. A. Long[1], [1]*Beltsville Agricultural Research Center*, *Beltsville, MD*, [2]*Hy-Line International*, *Dallas Center, IA*.

The fertility rates of cryopreserved poultry semen are highly variable and not reliable for use in commercial production or preservation of genetic stocks. Our objective was to evaluate the cryosurvival of semen from 8 pedigreed layer lines at the onset and end of production. Semen from 160 roosters (20/line) was frozen individually with 11% glycerol at 6 and 12 mths of age. Glycerol was removed from thawed semen by Accudenz gradient centrifugation. The viability of thawed sperm from each male was determined using SYBR/PI and flow cytometry. The fertilizing ability of thawed sperm was evaluated in vitro by assessing hydrolysis of the inner perivitelline layer. Hydrolysis data were grouped in 3 categories: <20 holes/mm^2; 21-80 holes/mm^2; and >81 holes holes/mm^2. Viability data were compared among lines and between age groups by repeated measures ANOVA. For hydrolysis data, logistic regressions were calculated to predict the natural log of the odds for males within lines to be in 1 of the 3 categories. The percentage of live sperm increased (p<0.0001) with age (6 mths, 35.6±0.8; 12 mths, 41.4±0.7%) and differed (p<0.0001) among lines, with 1 line consistently superior at both 6 and 12 mths. Hydrolyzing ability increased (p=0.0003) from 6 (54.5±6.1 holes/mm^2) to 12 mths (96.7±9.4 holes/mm^2) and differed among lines, with the odds of sperm hole category dependent upon line and age. Overall, viability was correlated with sperm hole number (r=0.24, p<0.0001) and category (r=0.25, p=0.0004); however, when the hydrolysis data were split by line and/or age, only 1 line consistently was correlated at both ages. These results demonstrate variability among pedigreed lines in withstanding glycerol-based semen cryopreservation. Further delineation of genotypic and phenotypic factors impacting sperm cryosurvival will be investigated in these genetic stocks.

Key Words: Chicken, Freezing, Fertilizing Ability

W235 Transcript profiling in mammary of ovariectomized pregnant gilts receiving progesterone and relaxin replacement therapy in late gestation. D. E. Graugnard*, J. J. Loor, E. A. Cutler, R. E. Everts, S. L. Rodriguez-Zas, and W. L. Hurley, *University of Illinois*, *Urbana*.

Relaxin-family peptides appear to have been important for the evolution and adaptation of lineage-specific physiologic processes (e.g.

lactation) during evolution. We previously determined that relaxin has a stimulatory effect on growth of mammary parenchymal tissue during late gestation (d 80-110) in the pig (Endocrinol. 128:1285-1290). To explore mammary genomic adaptations elicited by relaxin we used a swine oligoarray (70 mer) with 13,263 functional hits for transcript profiling of mammary tissue (n = 5/group) from control gilts sacrificed on d 110 of gestation (C110), gilts ovariectomized on d 100 and receiving progesterone (OP110), and gilts as in OP110, but also receiving porcine relaxin (OPR110). Annotation of the array was based on similarity searches using BLASTN and TBLASTX against human, mouse, and pig UniGene databases, the human genome, and pig TIGR. Cy3- and Cy5-labelled cDNA from mammary tissue and a reference standard were used for hybridizations. ANOVA ($P \leq 0.05$) identified 337 differentially expressed genes with OP110 or OPR110 vs. C110. Among these, there were 111 downregulated genes (ANGPT2, TAF12, NR1D2) by ≥2-fold with OP110 or OPR110 vs. C110. The extent of downregulation vs. C110 was more pronounced with OP110 (2-to-24-fold) than OPR110 (2-to-4-fold). Relaxin replacement therapy partially corrected downregulation of genes elicited by ovariectomy as shown by differences in expression between OP110 and OPR110, e.g. 102 genes with 2-fold (E2F5, AP2M1, TP53BP1) to 13-fold (LALBA, CSN1S2A, ANGPT2) upregulation due to OPR110 vs. OP110. Ingenuity Pathway Analysis of ≥2-fold upregulated genes with C110 and OPR110 vs. OP110 showed that molecular transport (19 genes), cell signaling (15 genes), cell growth/proliferation, and carbohydrate/lipid metabolism (10 genes in each) were among the most significant families of related genes. Overall, results demonstrate previously unrecognized genomic adaptations in the mammary gland elicited by relaxin.

Key Words: Mammary Gland, Relaxin, Micro Array

W236 Effect of boron supplementation on semen quality in mature boars. W. L. Flowers*[1], J. W. Spears[1], and F. H. Nielsen[2], [1]*North Carolina State University*, *Raleigh*, [2]*USDA-ARS, Grand Forks Human Nutrition Center*, *Grand Forks, ND*.

The objective of the study was to determine the effect of dietary boron on sperm production and semen quality in mature boars. Twelve crossbred boars (Landrace × Large White × Duroc × Hampshire) that were 2.5 ± 0.2 years of age and 215 ± 5 kg were randomly assigned to receive 0, 25, or 250 mg per head per day of supplemental boron for 8 weeks (n=4 per treatment). Boron was added to their basal diets and boars were fed 2.75 kg daily of a corn-soybean meal diet (14% crude protein). Concentrations of boron in the whole semen and seminal plasma fractions of ejaculates collected at the end of 7 weeks were different among treatments ($P<0.05$). The highest and lowest levels in semen and seminal plasma were measured in boars receiving 250 (708.7 and 692.0 ng/ml) and 0 mg of supplemental boron (88.9 and 76.5 ng/mL), respectively, while concentrations in boars receiving 25 mg (148.0 and 145.4 ng/mL) were in between the other two treatments. No effect of time or treatment on semen volume ($P>0.70$); total number of spermatozoa per ejaculate ($P>0.82$); or the proportion of spermatozoa exhibiting progressive forward motility ($P>0.89$) was observed. There was a tendency for a time by treatment interaction ($P=0.09$) for the proportion of spermatozoa with normal morphology. Normal morphology averaged 72.0% for control boars and did not change over time ($P>0.89$). In contrast, it tended to increase ($P<0.10$) from 74.7% and 76.1% during the first week of the study to 86.4% and 88.2% during week 8 in boars fed 25 and 250 mg of boron, respectively. Average path, straight line, and curvilinear velocities of motile spermatozoa increased over time ($P<0.05$) in boars receiving supplemental boron, but remained constant ($P>0.45$) in control boars (treatment × time; $P<0.05$). In conclusion, supplemental dietary boron enhanced sperm velocity characteristics and possibly, normal morphology, without influencing other measures of sperm production and semen quality.

Key Words: Boars, Boron, Spermatogenesis

W237 Transient transgene transmission to piglets by sperm-mediated gene transfer. Z. Wu[1], Z. Li[1,2], and J. Yang*[2], [1]*South China Agricultural University*, *Guangzhou, Guangdong, China*, [2]*University of Hawaii*, *Honolulu*.

An efficient and low-cost production of transgenic pigs has significant applications to the pig industry and biomedical science. Generation of transgenic pig by sperm-mediated gene transfer was inexpensive and convenient, but reported with inconsistent results. To test the method of sperm-mediated gene transfer in pigs, we employed deep post-cervical intrauterine insemination of incubated spermatozoa in this study. A test of sperm motility of semen from nine Landrace boars after incubation with radioactively-labeled DNA construct indicated that DNA uptake of the sperm were highly correlated with sperm motility at the time of collection. DNA concentration of 50 and 300 μg per one billion sperm was incubated with washed sperm at 17°C for 2h. Twenty-one hybrid gilts and sows of Meishan crossed with Large White were inseminated with transgene-incubated sperm and produced 156 piglets. Transgene DNA sequences were identified in 31 piglets by PCR amplification of genomic DNA isolated from piglet ears at the age of 3 days. The deep intrauterine insemination had a higher rate of positive transgenic piglets than regular insemination (29.6% of 98 piglets vs 3.4% of 58 piglets). However, the exogenous transgene DNA was not detected in all the piglets at the age of 70 to 100 days. Therefore, the results further demonstrated that that transgene through incubation with sperm was mostly transiently transmitted to the offspring at early growing stage and lost in adulthood, which may result from episomal DNA replications during germ cell division.

Key Words: Transgenic, Sperm-Mediated Gene Transfer, Episome

W238 Computer-assisted analysis of sperm parameters after selection of motile sperm by either percoll gradient, filtration or swim-up procedures. C. N. Person*, T. D. Lester, M. D. Person, and R. W. Rorie, *University of Arkansas*, *Fayetteville*.

A Hamilton-Thorne IVOS sperm analyzer (CASA) was used to compare sperm parameters of frozen-thawed semen from 10 bulls, before and after selection of motile sperm by either percoll gradient, filtration or swim-up procedures. Semen was thawed, washed in TALP medium and initial sperm parameters measured before assignment across selection methods. Sperm parameters measured at 0, 4 and 8 h after selection included motility, progressive motility, velocity distribution, path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), beat frequency (BCF), straightness (STR), linearity (LIN), elongation and area. Percoll separation resulted in more total motile sperm recovered than either swim-up or filtration ($P=0.00$), while swim-up did not differ from

filtration (P=0.31). Percent motile, progressive, and rapid sperm did not differ (P=0.87, 0.91, and 0.94, respectively) across treatments. Across bulls, the percent motile sperm declined by ~50% from 0 to 4 h of culture, and by another ~33% from 4 to 8 h of culture. The VAP and VSL were similar (P≥0.09) across treatments, whereas VCL was greater (P=0.00) for both filtration and swim-up than for percoll. Sperm LIN and STR were similar across treatments (P=0.24 and 0.89). The ALH was greater for filtration and swim-up than for percoll (P≤0.01). The BCF for filter selected sperm was greater than that of either percoll or swim-up (P≤0.00). Sperm head elongation and area were also greater for filter selected than either swim-up or percoll (P≤0.00), whereas these parameters were similar for percoll and swim-up (P≥0.68). Based on total motile sperm recovered, percoll separation is superior to the other methods. Overall, results suggest that the method used for selection of motile sperm can influence some of the parameters related to motility and sperm head morphology. Further study is needed to determine if these differences are related to fertilization rate or developmental competence after IVF.

Key Words: Bovine, Sperm Parameters, CASA

Production, Management & the Environment - Livestock and Poultry III

W239 Effect of soaking dairy cows at the feed line on animal body temperature in a tunnel ventilated barn equipped with evaporative pads located in a tropical climate, Thailand. D. V. Armstrong*[1], M. J. VanBaale[1], S. Rungruang[2], V. Wuthironarith[2], M. J. Brouk[3], and J. F. Smith[3], [1]*The University of Arizona*, *Tucson*, [2]*Charoen Pokphand Group Co., Ltd.*, *Bangkok, Thailand*, [3]*Kansas State University*, *Manhattan*.

An experiment was conducted on ten lactating Holstein cows to evaluate the effect of soaking dairy cows at the feed line. The cows were housed in a two row tunnel ventilated free stall barn equipped with evaporative pads and a feed line soaker system. The free stall barn is 16 m by 113 m with a ceiling height of 2.6 m. The barn is equipped with 55.7 sq m of 2.4 cm thick evaporative pads on one end and eleven 130 cm fans on the opposite end of the barn. Air speed in the barn at animal shoulder height averages 9.7 km per hour. Air exchange is every 42 sec. Two treatments were utilized in this experiment: no feed line water spray (C) and feed line spray (FLS) from 1100 to 0600. Treatments were reversed every 4 days in a 2 × 2 Latin square design. The soaker cycle was 1 minute on and 4 minutes off. Water application was 2.8 liters of water per cow per cycle. Nozzles are located every 1.87 m on the line located at a height of 1.6 m from the floor. The average ambient temperature was 29.1°C, with relative humidity (RH) at 68%, and a temperature humidity index (THI) of 79. The average temperature inside the barn was 25°C, with the RH at 91% and a THI of 78. Individual cows were fitted with stainless steel temperature data loggers that recorded their core body temperature (CBT) at five-minute intervals throughout the study. Average CBT for the control group was higher, 39.08°C, than the cows with FLS, 38.99°C and is significant (P<0.01). The results of this trial suggest that feed line soaking has an additive effect for cooling cows in a tunnel ventilated barn located in a tropical climate. The difference between treatment CBT was from 06:00 to 09:00 when the ambient relative humidity is the highest and the difference between ambient and barn temperature is the lowest.

Key Words: Body Temperature, Feed Line Cooling, Tropical Climate

W240 Effect of soaking dairy cows at the feed line on animal behavior in a tunnel ventilated barn equipped with evaporative pads located in a tropical climate, Thailand. D. V. Armstrong*[1], M. J. VanBaale[1], S. Rungruang[2], V. Wuthironarith[2], M. J. Brouk[3], and J. F. Smith[3], [1]*The University of Arizona*, *Tucson*, [2]*Charoen Pokphand Group Co., Ltd.*, *Bangkok, Thailand*, [3]*Kansas State University*, *Manhattan*.

An experiment was conducted on ten lactating Holstein cows to evaluate the effect of soaking dairy cows at the feed line. The objective of the study was to observe if there are any changes in animal behavior for dairy animal that are soaked at the feed manger for 19 hours per day. The cows were housed in a two row tunnel ventilated free stall barn equipped with evaporative pads and a feed line soaker system. The free stall barn is 16 m by 113 m with a ceiling height of 2.6 m. The barn is equipped with 55.7 sq m of 2.4 cm thick evaporative pads on one end and eleven 130 cm fans on the opposite end of the barn. Total air exchange for the barn takes place every 42 sec. Treatments were no feed line water spray (C) and feed line spray (FLS) from 1100 to 0600 (19 hours). Treatments were reversed every four days in a 2 X 2 latin square design. The soaker cycle was 1 minute on and 4 minutes off. Water application was 2.8 liters of water per cow per cycle. Nozzles are located every 1.87 m on the line, which is at a height of 1.6 m from the floor of the feed line. Cow behavior data was collected every 15 minutes for 24 hours (96 observations per cow) over a 5 day period. Each pen of cows was on the C or FLS treatment for 5 days and then the groups were reversed. Twenty-four hour cow observation took place on day 5 of each period. The results would indicate three of the measurements of dairy animal behavior were changed with the addition of a feed line water soak for 19 hours per day. The time that cows spent eating was increased from 14.9% in the control group to 16.7% for the FLS. Standing time at the feed line was also increased to 6.2% for FLS compared with 4.4% for C. Time spent at the water trough was higher for C at 4.9% than 3.3% for FLS. All other observations were not measurably different.