M287 Endocrine events during the periestrus period and the effect of various PMSG on estrus synchronization in shall ewes. T. Saberifar, H. Kohram*, and E. Dirandeh, University of Tehran, Karaj, Tehran, Iran.

The aim of the present study was to investigate the endocrinology of periestrus period and the days after until estrus in ewes (n = 40) synchronized out of breeding season and diagnosing the related pregnancies after laparoscopic insemination comparing to different doses of PMSG that was used. Animals treated for 12 d with CIDR followed by administration with different doses (0, 450, 550, 650, 750, 850 IU) of PMSG after CIDR withdrawal. Number of ewes in each group was 7 except for control group that was 5. 48 h after CIDR removal, ewes were inseminated by frozen semen using laparoscopy method. Blood samples were collected once daily from the jugular vein into heparinized tubes beginning at day CIDR removal until the day of estrus. Plasma was harvested and stored at –20°C until progesterone or estradiol assay. Concentrations of progesterone (Progesterone RIA, Biosource, Belgium) and estradiol (estradiol RIA, Biosource, Belgium) were determined. Pregnancies were diagnosed using ultrasonography at d 30 following in ewes. Progesterone levels did not differ between groups during periestrus period. Estradiol levels also did not differ between groups during the days CIDR removal and one day later. However, estradiol levels in the control group compared to groups 550 and 850 was significantly lower and higher (control: 12.14 pg/mL, 550:19.91 pg/mL, 850:8.98 pg/mL) at estrus, respectively. Pregnancy diagnosis showed that, the number of nonpregnant ewes was lower in group 550 and higher in group 850 comparing to control group (control: 3, 550:0, 850:4). The result of this study demonstrated that 550 IU PMSG would be the best dose to increase the plasma estradiol at the onset of estrus and also for synchronizing Shall ewes out of breeding season and have been inseminated by frozen semen using laparoscopy method. Blood samples were collected once daily from the jugular vein into heparinized tubes beginning at day CIDR removal until the day of estrus.

Key Words: periestrus, PMSG, shall ewes


To determine the effect of bypass fat in serum concentrations of luteinizing hormone (LH), estradiol (E2), progesterone (P4) and insulin (INS) during 7d before removal of intravaginal sponges in Dorset ewes, ewes (n = 59) were classified by thickness of dorsal fat by ultrasonography with a 7.5-MHz transducer. Ewes with low (LDF, 2mm) and high (HDF, 4mm) thickness dorsal fat were subdivided in groups: Low thickness without (LDF0, n = 14) and with (LDF1, n = 14) the addition of 150 g of bypass fat; High thickness without (HDF0, n = 15) and with (HDF1, n = 16) the addition of 150 g of bypass fat, respectively. Estrous cycles were synchronized with sponges of flugesterone acetate (FGA, 20 mg), for 12 d, 10 d after insertion 15 mg of prostaglandins (PGF2α) were injected. LH was analyzed by PROC GLM and means by Tukey; P4, E2 and INS by PROC MIXED and mean values by least squares means (SAS). There were no differences (P ≥ 0.05) by addition of fat, in onset, duration, and LH preovulatory peak, however, amplitude of LH preovulatory peak was different among treatments (P ≤ 0.05). P4 concentration was higher in ewes without addition of fat compared with the added group (P ≤ 0.05, 2.74 ± 0.2 vs. 2.58 ± 0.2 ng mL−1). E2 and INS concentrations increased in ewes with HDF compared with LDF group (P ≤ 0.05, 15.4 ± 11.8 pg mL−1, 0.37 ± 0.02 pg mL−1 vs. 5.1 ± 11.8 pg mL−1, 0.25 ± 0.02 pg mL−1, respectively). It is concluded that the addition of bypass fat did not alter onset and duration of LH preovulatory peak, but decreased P4 concentration probably due to an early secretion of PGF2α, nevertheless, E2 and INS concentrations increased in ewes with HDF, which is attributed to an improved metabolic, nutritional and body animal status.

Key Words: ultrasonography, hormones, synchronized estrus.


This study was conducted to determine if multiple injections of human chorionic gonadotropin (hCG) will increase circulating concentrations of progesterone (P4) in sheep following mating and prolong elevated levels through the period of fetal attachment. Fifty-nine nulliparous, primiparous, and multiparous Suffolk ewes (avg BW = 79.7 ± 2.5 kg) received an intravaginal P4-containing insert (CIDR, 0.3 g P4) for 12 d and were mated with fertile rams on the second estrus after CIDR removal. Ewes were randomly assigned to one of 2 treatments. The treated group received 200 IU (0.4 mL) of hCG im and controls received 0.4 mL saline im on d 4, 7, and 10, after onset of estrus (d 0; mating). Blood samples were taken via jugular venipuncture beginning on d 0 and on alternate days until d 35. Serum P4 concentrations were similar (P > 0.10) between treatment groups through d 5. However, beginning on d 7, ewes treated with hCG had greater (P < 0.01) serum P4 concentration than controls, and P4 remained higher (P < 0.05) throughout the sampling period (d 35). Of ewes receiving hCG, 68% had 4 or more total CL present compared with 33% for controls (P < 0.05; determined by laparoscopy on d 25). Fetal numbers were determined via flank ultrasonound on d 60 and 85% of hCG-treated ewes had multiple fetuses compared with 62% of controls (P < 0.10). In addition, 82% of hCG-treated ewes gave birth to 2 or more lambs compared with 63% of control ewes (P = 0.17). In conclusion, hCG administration on d 4, 7, and 10 after mating resulted in elevated serum P4 concentrations from d 7 through d 35, with more ewes carrying multiple fetuses.

Key Words: hCG, progesterone, lamb crop

M290 Administration of genistein does not alter anterior pituitary concentrations of LH and IGF-I in ovariectomized gilts. C. Paulson*, A. Taylor, and J. Clapper, South Dakota State University, Brookings.

Previously we have shown that administration of genistein to barrows increases anterior pituitary (AP) concentrations of IGF-I and LH and increased expression of AP IGF receptor. However, whether similar changes occur in ovariectomized gilts remains to be determined. Therefore, the objective of this experiment was to determine if short-term administration of genistein alters serum and anterior pituitary (AP) concentrations of LH and IGF-I and expression of AP GnRH receptors and LHβ subunit. Sixteen cross bred gilts of similar weight (95.5 kg)
were ovariectomized and assigned to either control (C; n = 8) or genistein (G; n = 8) groups. G pigs received 800 mg of genistein in DMSO while C pigs received an equal volume of DMSO i.m. on d 0, 1, 2, and 3. Blood samples were obtained by jugular venipuncture on d 0, 1, 2, and 3. Pigs were slaughtered on d 4 when blood and AP were collected. Serum and AP concentrations of LH and IGF-I were determined in duplicate by RIA. Relative expression of GnRH receptor and LHβ subunit were determined by real time RT-PCR, and fold changes analyzed using the REST software. Differences in serum and AP concentrations of LH and IGF-I were determined using the PROC Mixed procedure of SAS. Serum concentrations of LH were not different (P > 0.05) in G pigs compared with C pigs on d 0, 1, 2, 3 and 4. Serum concentrations of IGF-I were not different (P > 0.05) between C and G pigs on d 0 through d 4. AP concentrations of LH and IGF-I did not differ (P > 0.05) between C and G pigs. Relative expression of LHβ subunit and GnRH receptor did not differ (P > 0.05) between C and G pigs. These preliminary data suggest that short-term administration of genistein does not increase serum and AP concentrations of LH or IGF-I, or expression of GnRH receptors and LHβ subunit in the ovariectomized gilt.

Key Words: genistein, IGF, pigs

M291 Changes in plasma concentrations of growth hormone and luteinizing hormone in ewes following central and peripheral treatment with kisspeptin. B. K. Whitlock1,2, J. A. Daniel1, B. P. Steele1, and J. L. Sartin1,2, 1Department of Large Animal Clinical Sciences, College of Veterinary Medicine, The University of Tennessee, Knoxville, 2Department of Animal Science, Berry College, Mt. Berry, GA, 3Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL, 4Agricultural Experiment Station, Auburn University, Auburn, AL.

Kisspeptin (KP), a neuroendocrine regulator of gonadotropin releasing hormone, has been hypothesized as an integrator of nutrition and hormones critical to metabolism and regulation of reproduction. Recent evidence suggests growth hormone (GH) secretion may be influenced by KP. The objective of this study was to determine if the GH stimulatory effect of KP is due to actions on the hypothalamus or anterior pituitary gland in ewes. Adult ovariectomized ewes (n = 8) were fitted with intracerebroventricular (ICV) cannula to facilitate central administration of experimental treatments. Ewes received one of 8 treatments [4 intravenously (IV) and 4 ICV]. Peripheral treatments [0 (Veh), 100, 200, or 1000 pmol/kg body weight (BW) KP-10 (human KP 45–54; 4389-v, Peptide Institute, Inc., Osaka, Japan) in saline] were administered as a bolus via jugular catheter and ICV treatments [Veh, 50, 100, or 200 pmol/kg BW KP-10] were administered via the ICV cannula. Blood samples were collected from a jugular catheter at −15, 0, 10, 20, 30, 45, 60, and 75 min relative to treatments. Experiments were repeated until all ewes received each treatment. Plasma GH and luteinizing hormone (LH) concentrations were determined by radioimmunoassay. Effects of treatment on plasma concentrations of LH and GH were tested using repeated measures (SAS Institute Inc., Cary, NC). The 200 and 1000 pmol/kg IV KP-10 increased (P < 0.05) plasma concentrations of LH. However, there was no effect of IV KP-10 on plasma GH. Conversely, 100 and 200 pmol/kg KP-10 administered ICV increased (P < 0.05) plasma GH concentrations. Maximum GH responses occurred 30 min following ICV KP-10 injection and were greater (P < 0.05) than both Veh and the 50 µg/ml KP-10 ICV. In addition to activating the gonadotropic axis, KP can activate the somatotropic axis in ruminants and present data support a central site of action.

Key Words: sheep, kisspeptin, growth hormone

M292 Temporal changes during the periparturient period on metabolic and endocrine parameters of spring-calving beef cows in grazing conditions. A. L. Astessiano1, R. Pérez-Clariget1, G. Quintans1, P. Soca1, and M. Carrquiry1, 1School of Agronomy, Udelar, Uruguay, 2Instituto Nacional de Investigación Agropecuaria, Treinta y Tres, Uruguay.

Primiparous crossbred cows (Hereford/Angus, n = 20), classified by BCS at calving (scale 1–8), were used in a randomized block design, to study temporal changes on metabolic and endocrine parameters during the periparturient period in grazing conditions. Cows grazed together a native pasture paddock (60 ha) with an average forage mass available of 453 kg DM/ha (13.2% CP, 24.4% ADF). Means from repeated measure analyses were considered to differ when P < 0.05. Cows BCS throughout the periparturient period evaluated was greater for BCS > 3.75 than BCS ≤ 3.5 calved cows. Concentrations of NEFA were affected (P < 0.01) by day postpartum (DPP) as NEFA were elevated at −45 DPP, peaked at −7 DPP and were reduced thereafter for all cows. However, NEFA levels were less from −45 to −15 DPP but greater at −7 DPP for BCS > 3.75 than BCS ≤ 3.5 calved cows, while no differences between groups were observed during the postpartum. Glucose, urea, and total protein concentrations were affected (P < 0.01) by DPP and there was an interaction (P < 0.03) between BCS at calving and DPP for urea and total protein levels. Glucose concentrations tended (P = 0.07) to decrease from −7 to 30 DPP while urea concentrations were elevated at −45 DPP, peaked at −7 DPP and decreased thereafter. However, changes in urea were steep around parturition for BCS > 3.75 but not for BCS ≤ 3.5 calved cows. Total protein concentrations were elevated during the last month of gestation, decreased at parturition and remained reduced until 45 DPP. Cows with BCS > 3.75 at calving had greater total protein at −15 DPP but less around parturition than cows with BCS ≤ 3.5. There were no differences in circulating insulin levels along the period evaluated, however, insulin was less for BCS > 3.75 than BCS ≤ 3.5 calved cows dueto a drastic increase of insulin levels after 15 DPP in the lastest ones. Metabolic and endocrine parameters reflected the negative energy balance during the prepartum of cows grazing native pastures in winter.

Key Words: peripartum, metabolites, cattle
and change in creatine from d1PF to wn were -ASSOC (P < 0.05) with pwE in 2nd parity sows only. 1st parity WEI was -ASSOC (P < 0.05) with d1PF creatine and +ASSOC (P < 0.05) with change in creatine from d1PF to wn. OR was +ASSOC (P < 0.05) with LEA at wn of 1st parity sows whereas OR in 2nd parity sows was +ASSOC (P < 0.05) with wn wt. Creatine at wn in 1st parity sows was -ASSOC (P<0.05) to both wn wt and LEA at wn. Backfat in 1st parity sows was associated with pwE whereas creatine at wn in 2nd parity sows was coupled with pwE. First parity WEI was related to creatine at d1PF, which may reflect stress events associated with parturition. These data suggest that body wt, bft, LEA, and creatine contribute to the complex trait of pw reproduction but may respond differently dependent upon female maturity.

**Key Words:** swine, reproduction, metabolism

**M294** Lipoic acid decreases progesterone clearance rates in ovariectomized ewes.  R. S. Mottet*, 1, C. O. Lemley, 2, E. L. Berg, 1, E. P. Berg, 1, and K. A. Vonnahme, 1; 1North Dakota State University, Fargo, 2West Virginia University, Morgantown.

Lipoic acid is a naturally occurring compound that has been shown to modulate insulin sensitivity when supplemented to the diet. Elevated blood insulin concentrations have been shown to decrease progesterone catabolism in several species by modulating expression and/or activity of cytochrome P450 2C (CYP2C) or 3A (CYP3A). We hypothesized that lipoic acid would decrease progesterone catabolism by the liver. The objective was to determine how supplementation of lipoic acid impacted progesterone clearance rate in ovariectomized ewes. Eight ovariectomized ewes were fed an alfalfa-grass ration at 95% of ad libitum for the duration of the experiment. Ewes were randomly assigned to lipoic acid treatment [an empty bolus administered by gavage (n = 4; CON), or lipoic acid supplemented at 32 mg/kg BW administered by gavage (n = 4; LA)]. Progesterone was administered via CIDR devices on d 5 to all ewes. Daily blood samples were collected from d 5 to 10. On d 10, liver biopsies were obtained from each ewe to determine CYP2C and CYP3A activity. On d 11, serial blood samples were collected after CIDR removal to determine progesterone clearance from the blood stream. Ewes treated with LA had decreased (P < 0.03) serum progesterone clearance compared with CON ewes; however, no difference (P > 0.20) in hepatic enzyme activity was found. We conclude that while lipoic acid decreases progesterone clearance in the blood, it does so without affecting hepatic CYP2C or CYP3A enzyme activity; therefore, the mechanism of action is yet to be elucidated.

**Key Words:** lipoic acid, progesterone, liver


Zearalenone (zea) is a potent mycotoxin that has estrogenic properties. In vitro results indicate that zealand metabolites are capable of downregulating proteins associated with protein synthesis (mammalian target of rapamycin, mTOR) and cellular proliferation (extracellular signal-regulated kinase, ERK) in muscle. The objective were to determine the effect of zea consumption by prepubertal gilts on: 1) growth performance, 2) reproductive tract development, and 3) skeletal muscle mTOR and ERK activation. Gilts were weaned at 21 d of age and allowed to adjust for 1 wk on a commercial diet. After 1 wk (d 0), gilts were randomly assigned to consume a commercial basal diet (C, n = 9) or C + 1.5 mg/kg zea (n = 10) for 4 wk, at which time gilts were killed, urine collected, and tissue collected and frozen. Zearalenone, α-zearalenol, and β-zearalenol were detected at levels of less than 4 µg/kg in urine of C gilts, but were increased (292 ± 76, 113 ± 20, and 15 ± 3 µg/kg, respectively) in pigs consuming zearalenone (P < 0.01). No differences were observed in ADG, ADFI, or G:F between treatments (P > 0.28). Reproductive tract size was increased 1.5-fold (20.9 ± 4.3 vs. 50.6 ± 3.8 g) in zea gilts (P < 0.01). Uterine endometrial gland development was increased 50% in gilts consuming zea (P < 0.01). In uterus, estrogen receptor (ER)-α mRNA and protein were unchanged (P > 0.25), but gilts consuming zea had 2- and 3-fold higher abundance of ER-β mRNA and protein, respectively, compared with the C group (P < 0.01). No differences were observed in mTOR and ERK protein phosphorylation or total abundance in skeletal muscle (P > 0.36). The consumption of zea had no effect on growth performance or skeletal muscle signaling in pubertal gilts, but zea increased reproductive tract size and glandular development, possibly due to altering the expression of ER-β.

**Key Words:** swine, uterus, zearalenone

**M296** Quantitative bioluminescence imaging of porcine antral follicles in vitro.  S. Jung* and S. T. Willard, Mississippi State University, Mississippi State.

Determining the relationships between optimal imaging time, plasmid DNA dose and luciferase expression for quantitative bioluminescence imaging is critical to the development of new biophotonic paradigms. In this study, we analyzed the time course of luminescence emitted from transfected porcine whole follicle units. We used cationic lipid transfection with increasing doses of plasmid DNA (pGL4) encoding a luciferase reporter gene. Follicles between 5.5 to 6 mm in diameter were dissected from the ovaries. DNA–lipid complexes were formed at a DNA (µg):lipid (µL) ratio of 2:5 in PBS, by adding FuGene 6 diluted in PBS to 0 µg, 1 µg, 2 µg or 3 µg of pGL4 and introduced into each follicle unit by injection using a microinjector. A total of n = 48 follicles were randomly allocated in 4 groups (negative control, 1 µg, 2 µg and 3 µg groups) and cultured at 39°C under 45% O2; 50% N2; 5% CO2 for 20 h. The luminescence from each follicle unit was detected using an IVIS 100 imaging system after 300 µg of luciferin was injected. An imaging series of 30-s exposures were collected up to 10 min. The signal intensity was reported as mean ± SEM of photon counts (PC; n = 12 per group), and the experiment was repeated 6 times. Data were analyzed by repeated measures ANOVA. The means of the negative control group at individual peak time points served as a background signal and subtracted from means of 1 µg, 2 µg, and 3 µg groups. The signal intensity reached a peak at 1.5 min (1.08 ± 0.47 × 105 ± 0.47 × 105 PC) in the 3 µg group, and at 1 min in both 1 µg (2.45 × 103 ± 0.88 × 103 PC) and 2 µg (4.60 × 102 ± 1.62 × 102 PC) groups after the injection and declined gradually afterward. The luciferase expression level of follicles in the 3 µg group tended to be greater than the 1 µg group (P = 0.09), but did not differ from the 2 µg group (P = 0.18). Overall, a higher level of luciferase expression was observed in follicles transfected with 3 µg of pGL4, with an optimal time for quantification at 1.5 min after luciferin injection. These data are the first to demonstrate luciferase detection and quantification, indicative of transcription, within whole antral follicles cultures in vitro.

**Key Words:** bioluminescence imaging, antral follicle, in vitro culture

M297  Feed restriction and pre-synchronization on progesterone concentration and LH peak in ewes on a synchronization program.  P. Molina1, T. Sánchez1, M. E. Ortega1, L. Olivares2, O.
To evaluate the effect of pre-synchronization and feed restriction in progesterone concentration and characterization of onset, duration and amplitude of LH peak, 69 Dorset ewes were randomly assigned to 4 treatments: Treatment 1 n = 18 (T1): Ewes received 1 kg of commercial supplement (16% protein) for 30 d and were synchronized with FGA sponges (40 mg) for 12 d; Treatment 2 n = 17 (T2) ewes received the same diet as T1, but they were synchronized with 2 doses of PGF<sub>2α</sub>, 16 and 8 d before sponges; Treatment 3 n = 17 (T3) ewes were feed restricted and received 1 kg of oat hay during 30 d, and synchronization regimen as T1; and Treatment 4 n = 17 (T4) received the same diet as T3 and the synchronization regimen as T2. Estrus was determined when ewes allowed the ram for mount after sponge removal. All groups showed 100% of estrus. Progesterone concentrations were greater in pre-synchronized groups (P ≤ 0.05) compared with non pre-synchronized. Feed regimen affected onset of LH peak (P ≤ 0.05), with a faster increase on groups with commercial supplement (T1 y T2), compared with groups feed with oat hay (T3 y T4). For synchronization program no differences were observed (P ≥ 0.05). Duration and amplitude of LH were not affected (P ≥ 0.05) for type of feed, synchronization program neither interaction between effects. Under the present experimental conditions, it is concluded that nutrition influenced onset of pre-ovulatory LH peak, while greater P4 concentration was observed in ewes pre-synchronized during the luteal phase, as a result of corpus luteum secretion.

**Key Words**: PGF<sub>2α</sub>, ewes, flugesterone acetate

**M298** Progesterone and insulin concentration on ewes with different body condition fed bypass fat in a superovulatory program. P. Molina<sup>1</sup>, T. Sánchez<sup>1</sup>, M. E. Ortega<sup>2</sup>, L. Oliva<sup>3</sup>res, O. Mejía<sup>3</sup>, M. Cárdenas<sup>4</sup>, E. García<sup>*5</sup>, J. Cordero<sup>1</sup>, J. Peralta<sup>6</sup>, and R. Nieto<sup>4</sup>, 1Programa de Ganadería, Colegio de Postgraduados, Texcoco, México, 2UAEM, Edo. México, 3CEIEPO, UNAM, Tres Marias, México, 4INNSZ, Mexico City, 5CUCSUR, Autlán, Jalisco, México, 6ICAP, UAEH, Hidalgo, México.

Dorset ewes in body condition score of 3 (1–5 scale) were randomly assigned to two treatments: In T1 (n=26) ewes were fed with commercial supplement and in T2 (n=21) ewes were fed with oat hay to reduce the body condition of these ewes and both groups received this diet for a month. Then six ewes of each group were superovulated (donors) and the rest remained as recipient ewes. At the beginning of the superovulation treatment dorsal fat was 2.5 and 1.97 mm and body weight was 69 and 65 kg, for T1 and T2, respectively. During the first 8 days of synchronization and superovulation treatment both groups received 100 g of protected fat and same diet as T1, synchronization for donors and receptors was performed by sponges of flugesterone acetate (FGA, 40 mg) during 12 days. Recipient ewes received 200 IU of eCG 12 h before sponge removal. Donor ewes were superovulated with decreasing doses of FSHp for 4 d and embryos were obtained and transferred 7 d later. At synchronized estrus ewes of T1 and T2 weighed 72 and 69 kg (P > 0.05) and dorsal fat measures were 3.5 and 3.29 mm (P > 0.05) respectively. All ewes from T1 (100%) responded to superovulation, while in T2 only 67% responded. Progesterone and insulin secretion were greater (P < 0.05) in ewes of the T1 group, compared to those of the T2 group. There were differences in number of corpora lutea present (9.5 vs. 14.7) for T1 and T2 groups, respectively, and also in rate of recovered embryos (93 vs. 71%). There were no differences in gestation rate (P > 0.05), with 35 and 31.5% for T1 and T2 groups, respectively. Based on the present experimental conditions, it is concluded that previous undernutrition affects ovulatory rate, and serum concentrations of progesterone and insulin but not pregnancy rate.

**Key Words**: FSH, embryos, Dorset ewes