POSTERS

P1 **ENDOCRINE AND OVARIAN RESPONSES ASSOCIATED WITH THE FIRST-WAVE FOLLICLE DOMINANT IN SHEEP SYNCHRONIZED EITHER CIDR OR $PGF_{2\alpha}$.** *E. Oba*, L. F. Uribe-Velásquez , L. C. Lara-Herrera*, M. I. L. Souza, H. Villa-Velásquez*, L. A. Trinca**, C. A. C. Fernandes.*** *Animal Reproduction and Radiology Veterinary Department, UNESP, 18618-000, Botucatu, SP, Brazil, *Faculty of Medicine, UNESP, 18618-000, Botucatu, SP, Brazil, **Biosciences Institute, UNESP, 18618-000, Botucatu, SP, Brazil and ***Faculty of Veterinay Medicine, UNIFENAS, 37130-000, Alfenas, MG, Brazil.*

The success of estrus synchronization programs using progestagens, particularly for fixed-time AI, varies considerably. In view of the recent evidence in cattle that exogenous progestins alter follicular dynamics, it may be that the stage of the estrous cycle at wich the synchronization protocol is begun affects the synchrony of ovulation (Leyva *et al.*, 1998; López Sebastián *et al.* 1999). The effect of PG vs CIDR and eCG (equine chorionic gonadotrophin) on dynamic follicular of wave 1 and its relationship with P_4 and E_2 plasma concentrations were investigated in cyclic ewes. The pattern of ovarian follicle development was characterized using the definition of a follicle wave as the changes in the number of follicles among the days of the estrous cycle (Evans *et al.*, 2000). Fourteen Bergamasca ewes were used, Group 1 (Control, G1) synchronized by two i.m. injections of PG and Group 2 (G2) was treated for 14 d with CIDR and an 500 IU of eCG at dispositive withdrawal on day 14. Ovarian follicular dynamics were ultrasonically monitored. Blood samples for P_4 and E_2 determination were collected daily from 1 d before the second injction of PG (G1) and administration of eCG (G2) until day 10 after ovulation of the cycle. The mean emergence day of wave 1 was 0.57 ± 0.53 vs 0.86 ± 0.40, respectively, for control and animals treated with CIDR + eCG. Diameter of the largest follicle of wave 1 in control animals (4.29 ± 0.26 mm) was less ($P<0.05$) than that of treated animals (5.36 ± 0.26 mm). The growth rate was different ($P<0.001$) among treatments with a mean of 1.0 ± 0.09 and 1.12 ± 0.07 mm/day for control and treated animals, respectively. A significant interaction was observed between treatment (CIDR + eCG versus control) and days (days 0-10) ($P<0.05$). Administration of eCG (500 IU) produced increasing of small follicles at the days –2 and –1 before ovulation and day 6 after ovulation, with values of 9.0 ± 1.21 vs 3.43 ± 0.30 ($P<0.001$); 8.43 ± 0.97 vs 4.29 ± 1.06 ($P<0.05$), and 8.43 ± 1.36 vs 4.43 ± 0.30 small follicles, respectively, for control and treated animals. There were significant ($P<0.01$) differences in P_4 and E_2 plasma concentrations among treatments. The CIDR + eCG (500 IU) treatment enhanced the recruitment of smaller follicles and enhanced the maximum diameter and growth rate of large follicles during wave 1 in Bergamasca sheep. Supported by FAPESP – São Paulo – Brazil.

References: Evans *et al.*, 2000, Theriogenology **53**, 699; Leyva *et al.*, 1998, *Theriogenology* **50**, 395; López Sebastián *et al.*, 1999, Theriogenology **52**, 505.

Key Words: follicular dynamics, progesterone (P_4), ultrasonography.

P2 **FOLLICULAR AND HORMONAL EVENTS ASSOCIATED WITH THE FIRST-WAVE DOMINANT FOLLICLE AFTER TREATMENT WITH PGF_2alpha IN SHEEP.** L. F. Uribe-Velásquez, E. Oba, M. I. L. Souza, L. C. Lara-Herrera*, H. Villa-Velásquez*, L. A. Trinca**, C. A. Price***. *Animal Reproduction and Radiology Veterinary Department, UNESP, 18618-000, Botucatu, SP, Brazil, *Faculty of Medicine, UNESP, 18618-000, Botucatu, SP, Brazil, **Biosciences Institute, UNESP, 18618-000, Botucatu, SP, Brazil, and ***Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, J2S7C6 Canada.*

Estrus synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency in sheep (Kusina *et al.*, 2000). However, the interval from treatment to estrus is variable, and is apparently affected by day of prostaglandin F_{2a} (PG) (Freitas *et al*, 1996, Viñoles & Rubianes, 1998). The purpose of this study was to evaluate ovarian response, using transrectal ovarian ultrasonography, to 2 injections of PG given at differents intervals. Fourteen Bergamasca ewes were used during the breeding season. Ewes were randomly allocated to two groups (n = 7) which received i.m. injections of a PG analogue 7d (G7) or 9d (G9) after ovulation (when the dominant follicle of wave 2 was expected to be in the growing and plateau phase, respectively). Ovaries were examined daily with transrectal ultrasonography Aloka 500 with 7.5. MHz linear array transducer. Ultrasonography started 1d day prior to the second PG injection and ended 10 d after ovulation for both groups. Jugular vein blood samples were collected daily for progesterone (P_4) assay. Mean maximum diameter size attained by the dominant follicle of wave 2 tended to be larger in the group treated in G7 when compared with G9 (5.5 ± 0.5 versus 4.29 ± 0.3 mm; $P<0.05$). The mean day of emergence of wave 1 was –0.7 ± 0.3 and 0.6 ± 0.5, respectively, for G7 and G9. Diameter of the largest follicle of wave 1 in G9 (4.3 ± 0.3 mm) was less ($P<0.05$) than that of G7 (5.4 ± 0.4 mm). The duration of the plateau phase was not different between G7 (1.9 ± 0.3 d) and G9 (1.3 ± 0.2 d). There was no difference in growth rates with values

respectively for G7 and G9 of 0.9 ± 0.5 versus 1.0 ± 0.1 mm/day. The proportion of large follicles increased (P<0.05) after luteolysis in G7. There were significant (P<0.0001) differences in P_4 among treatments. In conclusion, 1) the viable dominant follicle present at the time of luteolysis increased in diameter and became the ovulatory follicle, and 2) growth of dominant follicle of wave 1 and response to PG treatment were altered but no much different from the way were altered by luteal regression during natural estrus. Supported by FAPESP – São Paulo – Brazil.

References: Freitas *et al.*, 1996, Theriogenology 45, 1561; Kusina *et al.*, 2000, Theriogenology 53, 1567; Viñoles & Rubianes, 1998, Can. J. Anim. Sci. 78, 429.

Key Words: synchronization, Follicles, Ultrasonography

P3 EFFECT OF TWO DIFFERENT CO_2 LEVELS DURING LAST STAGE OF INCUBATION ON CARDIAC β-ADRENERGIC RECEPTOR CHARACTERISTICS OF BROILER CHICK EMBRYOS.

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Decreased oxygen tensions or increased oxygen requirements can create hypoxic conditions in the tissues which may in turn influence pulmonary vasomotor tone. Since the chick embryo consumes 60% more oxygen between the start of pulmonary breathing and hatching, compared to earlier stages, it is possible that a shortage of oxygen already occurs during this stage. Our previous studies showed that the binding capacity of myocardial β-adrenergic receptors of ascites-sensitive birds was higher compared to ascites-resistant birds (1). This experiment was designed to further elucidate to involvement of β-adrenergic receptors in the occurrence of ascites during the last atage of embryonic development. Six hundred forty eggs from a commercial broiler were incubated under standard conditions at 37.8 C and a relative humidity of 55 % in both forced-draught incubator. Eggs were equally divided over two incubators. From day 15 until day 20 the ventilation level in one incubator was decreased, resulting in a CO2 concentration of 0.40% whereas in the other incubator, the CO2 level was maintained at 0.20%. At the end of day 20, external pipping and hatching were recorded. Blood samples were taken at the stage of external pipping for analysis of blood gas parameters and haematocrit values. The thorax of embryos was opened and the heart was removed. The ventricles were used for analysis of β-adrenergic receptors as was described (1,2). When incubated under high CO2, hatching occurred earlier compared to normal CO2 levels. High CO2 embryos showed significantly lower pO2 (P< 0.05), higher pCO2 (P< 0.005) and higher haematocrit values (P< 0.0001) than normal CO2 embryos. These finding clearly indicate that high CO2 embryos were suffering from hypoxic conditions. Exposure of chick embryos to high CO2 levels reduced the binding capacity of myocardial β-adrenergic receptors (P<0.005) compared to those embryos incubated to normal CO2. Down-regulation of β-adrenergic receptor capacity observed in high CO2 embryos paralleled by the changes in haematocrit values, pCO2 and pO2 levels between two groups of chick embryos, suggesting the represent an adaptation mechanism of heart tissue to hypoxia as recorded earlier in mammals, cultured chick embryo ventricular cells and in hypertrophied and non-hypertrophied broiler chickens (1,2).

References: 1-Hassanzadeh et al. (1997). *Avian Pathology, 26, 293-303.*, 2- Hassanzadeh et al. (2001). *Avian Pathology, 30, Inpress.*

Key words: Ascites, chick embryos, β-adrenergic receptor

P4 PLASMA CONCENTRATIONS OF TRIIODOTHYRONINE, THYROXINE AND CORTISOL IN BEEF COWS IN CASCAVEL – PARANÁ– BRAZIL.

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Thyroid, a gland that influences diverse organic functions, utilizes iodine and tyrosine as integral part of hormones, thyroxine (T_4) and triiodothyronine (T_3; Toledo Neto *et al.*, 1991; Webster *et al.*, 1991). These hormones control metabolic activity of the body, regulate oxidative cellular processes and ribonucleic acid and plasmatic proteins synthesis, and are in their secretion affected by energy supply, interfering in oxygen consumption function, cellular ATP and heat production (Toledo Neto *et al.*, 1991). The cortisol produced by adrenal cortex since of cholesterol, participates in all metabolic aspects of regulation, directly or by interaction with other hormones, active neogligogenesis, proteic synthesis and lipolysis, and is involved in stress process (Cunningham, 1993). To knowledgement of endocrinologic behaviour of thyroid and adrenal glands in adults beef cows (of industrial

crossbreed), breed and maintened in Cascavel area, Paraná, Brazil, to study plasmatic levels of triiodothyronine, thyroxine and cortisol. 80 cows were utilized, blood collected by jugular venipunction in heparinized tubes, and plasma aliquoted and stored at -20°C. Plasma concentrations of triiodothyronine (T_3), thyroxine (T_4) and cortisol, were determined by RIA. Results were submitted at descriptive statistic (mean±SD). T_3 levels ranged between 53.34 and 242.3ng/dL, and mean 106.74±33.15 ng/dL, while T_4 levels ranged between 0.61 and 4.65µg/dL, and mean 2.44±0.83µg/dL. Cortisol presented mean of 3.07±1.85µg/dL, ranged between 0.41 and 7.59µg/dL. Mean levels revised by McDonald & Pineda (1989) were of 92.50-170.0ng/dL to T_3; 3.60-8.9µg/dL to T_4; and 4.46-4.54ng/mL to cortisol. From the analysis of these levels, we may conclude the high range in the incremented levels of T_4 and T_3, perhaps by abundant nutrition in pastages, and cortisol maintenance into mean normal levels for bovines, demonstrated absence of anabrupt stress response, by these animals, in the blood. Supported by IPEAC/UNIPAR – Umuarama – PR - Brazil.

References: Cunningham, 1993, 278; McDonald & Pineda, 1989, 202; Toledo Neto *et al.*, 1991, *Arq. Bras. Med. Vet. Zootec.*, 43, 489; Webster *et al.*, *Endocrinology*, 129, 176.

Key Words: beef cows, thyroid hormones, cortisol.

P5 RELATIONSHIP BETWEEN LARGE FOLLICLES AND ESTRADIOL LEVELS AT FLUSHING DAY AND EMBRYO RECOVERY IN BEEF HEIFERS AND COWS. C. A. C. Fernandes, E. Oba*, L. F. Uribe-Velásquez*, *Faculty of Veterinay Medicine, UNIFENAS, 37130-000, Alfenas, MG, Brazi,. *Animal Reproduction and Radiology Veterinary Department, UNESP, 18618-000, Botucatu, SP, Brazil,*

There is wide scope for the use of ultrasound as a tool to increase our understanding of bovine reproduction and to manipulate the reproductive processes to maximize the reproductive efficiency of this species (Ginther *et al.*, 1996). It may be possible to improve the response to superovulatory treatments by assessing follicular development ultrasonographically. A major limiting factor affecting widespread use of embryo transfer technology in bovine is the variable and unpredictable response to superovulation (Armstrong, 1993). This variability is often related to the day of the estrous cycle when superovulatory treatments are initiated. The major purpose of this study was to verify the effects of presence of large follicles at flushing day on estradiol levels and embryo recovery rate. Twenty five heifers and cows (Limousin breed) were superovulated by 240mg NIH-FSH-P1 (Folltropin – Vetrepharm Canada). The donors received 0.5mg of IM cloprostenol (Ciosin – Coopers Brazil) on 7th and 8th FSH injection. At flushing day evaluations of ovaries were performed by transrectal ultrasonography (Pie Medical 100LC – Pie Medical Co. Netherlands) and blood was collected for estradiol analysis. The number of corpora lutea and number and diameter of all follicles were analyzed. The recuperation rate (embryos/corpora lutea) from donors that showed at flushing day one or more follicles with diameter $\geq$12mm was lower than from donors that didn't show these follicles (37.42 $\pm$ 12.11 vs 65.13 $\pm$ 13.63; $P<0.05$). Those donors also showed higher estradiol levels (17.62 $\pm$ 4.57 pg/mL vs 10.82 $\pm$ 3.81 pg/mL; $P<0.05$). Estradiol from remaining follicles after the estrus may alter the cascade of endocrine and physiological events required to establish or maintain pregnancy in cattle. This estradiol may be increase oviductal contactions in contrary way to cilia beat of isthmic cells. These increase in the oviductal contractions can modify the embryo transportation through oviducts and decrease the embryo numbers that arrive to the uterus (D'Alessandro *et al.*, 1999). Though, the presence of large follicles that remain with steroidogenic ability from estrus until flushing day, are responsible for a hormonal disturbance that modifyes the embryo oviductal transportation physiology and embryo recovery rate. Supported by FAPESP – São Paulo – Brazil.

References: Armstrong, 1993, Theriogenology, **39**, 7; D'Alessandro et al., 1999, J. Reprod. Fertil., **115**, 185; Ginther et al.,1996, Biol. Reprod., **55**, 1187.

Key Words: estradiol (E_2), follicles, embryo transfer.

P6 GH SECRETION BY PIG PITUITARY CELLS : RELATIONSHIP BETWEEN LEPTIN AND NITRIC OXIDE . M. Baratta, R. Saleri, G.L. Mainardi, C. Tamanini. *Faculty of Veterinary Medicine, University of Parma, Via del Taglio 8, 43100 Parma, Italy.*

Recent studies suggest that leptin modulates growth hormone (GH) production by regulating both somatostatin gene expression and GHRH secretion by hypothalamic neurons. Since leptin receptors have been found in pituitary cells, a relationship between leptin and GH at this level has been suggested. Nitric oxide (NO) has emerged as an important intra- and intercellular transmitter involved in the control of the hypothalamic-pituitary axis and inducible NO synthase (iNOS) has been identified in the pituitary cells; furthermore, NO has been reported to be involved in GH secretion. The aim of this study was to investigate the direct effect of leptin on GH gene expression and GH secretion as well as the

role of NO as its mediator in pig pituitary cells. Anterior pituitary cells from adult sows were cultured in DMEM-F12 + 5 % FCS and treated for 4 or 24 h with rh-leptin (from 0.1 nM to 1μM), alone or in association with GHRP-6 (10 nM); cells were also treated for 24 h with S-nitroso-N-acetyl-penicillamine (SNAP), a NO donor, (0.1μM to 1 mM). In addition, N^G-Methyl-L-Arginine (NMMA, 300μM), a NO synthase inhibitor was associated with rh-leptin in the studies on NO output. At the end of incubations, medium was collected and GH and NO were determined by ELISA and Griess test, respectively. Total RNA was collected and GH gene expression was measured by RT-PCR. Semi-quantitative PCR was carried out using validated pGH primers. Rh-leptin significantly ($p<0.001$) stimulated GH secretion in both incubation periods. The maximum response was induced by rh-leptin 10 nM (4 h: 153.9±2.5 vs 65.9±2.3; 24 h: 566.7±6.8 vs 298.1±6.8 ng/ml, mean±SEM); furthermore, a significant interaction ($p<0.002$) between rh-leptin and GHRP-6 was observed. GH gene expression was increased up to 32% ($p<0.05$) by GHRP-6 and up to 78% and 136% by rh-leptin 1 μM and 0.1μM, respectively after 24 h of treatment. SNAP significantly ($p<0.05$) increased GH secretion even if GH increase was not related to NO released by the different SNAP concentrations ($p<0.05$). Rh-leptin 10 nM and 1μM significantly ($p<0.05$) stimulated NO production; this effect was reversed by a co-incubation of rh-leptin with NMMA. This study confirms a positive direct effect of leptin on GH mRNA levels and GH secretion by pig pituitary cells; this effect potentiates the GHRP-6-induced GH release; in addition, leptin is effective in stimulating NO production that, in turn, enhances GH secretion; furthermore, NO confirms to be an important intra- and intercellular transmitter involved in the regulation of the hypothalamic-pituitary axis. These results, taken together, support the hypothesis of a direct control of leptin on GH gene expression and secretion and, therefore, on the control of metabolic resources. This work was supported by a MURST COFIN grant.
Key words: GH, Leptin, Nitric Oxide, Pituitary Cells

P7 EVALUATION OF SUPEROVULATORY RESPONSE IN ANOESTROUS AND CYCLIC NATIVE COWS IN EGYPT WITH EMPHASIS ON HORMONAL AND BLOOD BIOCHEMICAL CHANGES. *Ahmed S. Abdoon*, W. M. Ahmed, Omaima M. Kandil and S. I. Shalaby. *Department of Animal reproduction & A.I. national Research Center, Dokki, 12622 Giza, Egypt.*

Ovarian status of the donor at the commencing of superovulation is one of the detrimental factors in superovulation regimen. The present study was carried out on 8 purebred native cows. Three superovulation regimens were conducted. In experiment 1, 8 non-cyclic cows received norgestomet ear implant, 3500 IU eCG on day 7 of implant application and 250 μg GnRH analogue during oestrus. In experiment 2, 6 cyclic cows received the same regimen with injection of 2500 IU eCG. In experiment 3, the same cyclic cows received the same treatment as in experiment 2, GnRH was replaced with 2000 IU hCG i.m. during oestrus. Timing to oestrus and duration of oestrus were recorded. Ovarian response was determined on day 7 post oestrus using rectal palpation and ultrasonography. Blood samples were collected on day-11, -4, -2, 0 and +7. Clear plasma was separated and stored at –20°C until analysis of P4 and T4 (RIA), cholesterol, total lipids, triglycerides, total protein, albumin, glucose and alkaline phosphatase (Spectrophotometery). The analysis of data showed that anoestrous cows displayed oestrous behavior at a significantly ($P<0.05$) longer time (52.00±2.53, 50.40±2.19 and 45.60±2.40 for experiment 1, 2 and 3 reps.), and significantly higher P4 level at implant removal ($P<0.01$) and during oestrus ($P<0.05$). Number of CL and non-ovulated follicles did not differ significantly between anoestrous and cyclic cows (5.80±0.80 and 6.00± 1.34; 7.60±1.31 and 5.20±2.13; 8.00± 1.34 and 3.40± 1.83 for exp. 1, 2 and 3 reps.). Plasma P4 levels reached their maximal values on day 7-post oestrus with a significant ($P<0.01$) lower value in experiment 1 compared with exp. 2 and 3. Plasma T4 level significantly ($P<0.01$) decreased in exp. 1 following treatment. Plasma P4 and glucose concentrations at eCG injection correlated significantly ($P<0.01$) with the subsequent ovulation rate. Plasma P4 correlated significantly ($P<0.01$) with the number of CL, glucose and cholesterol values. However, the correlation between other blood metabolites and either P4 or ovulation rate lacks significance. In conclusion, ovarian response to superovulation in native cows in Egypt is not affected by the ovarian status. Plasma P4 and glucose play a significant role in the subsequent ovulation rate. Other blood metabolites were of no value in the control of ovarian response to superovulation. Ultrasongraphy is more accurate in predicting the superovulation response than rectal palpation.
Key words: Reproductive status, anoestrous, superovulation, hormones.

P8 **EFFECT OF PRETREATMENT WITH MELATONIN ON THE OVARIAN RESPONSE, HORMONAL PROFILE, BLOOD BIOCHEMICAL AND CHROMOSOMAL CHANGES IN SUPEROVULATED BUFFALO HEIFERS.**

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The present experiment was set to study the effect of pre-treatment with melatonin on the ovarian response, P4, blood biochemistry and chromosomal aberration in superovulated buffalo heifers. Eight buffalo heifers were divided into 2 groups: Group 1 received no treatment and served as a control; Group 2 received silastic ear implant containing 100 mg melatonin (from June 30th to Aug 31st 1997). In mid Sept, both groups received norgestomet ear implant for 9 days. Superovulation was performed by injection of 2500 IU eCG on day 7 of implantation. During oestrus, 1000 IU hCG were injected i.m.; timing to oestrus, duration of oestrus were recorded. Ovulation rate was recorded on day-7 post oestrus using rectal palpation and ultrasonography. Blood samples were collected before eCG injection, during oestrus and on day-7 post oestrus for P4, T4 biochemical analysis and on day-7 for cytogenetical analysis. Results showed that timing to oestrus was shorter ($P<0.01$) and number of CL was higher ($P<0.01$) in melatonin treated buffalo heifers compared to control ones. A significantly higher number of CL and non-ovulated follicles was detected with ultrasonography than by rectal palpation. Serum P4 concentration was significantly higher in melatonin group before eCG injection ($P<0.05$) and on day-7 post oestrus ($P<0.01$) when compared with control one. Serum total lipids concentrations throughout superovulation and glucose on day-7 post oestrus were significantly higher in melatonin treated than in control group. Superovulation treatment significantly increased ($P<0.01$) chromosomal aberration and sister chromatid exchange (SCE) than before treatment in both groups. Also, the incidence of chromosomal aberrations and SCE were significantly higher ($P<0.01$) in control vs melatonin treated superovulated buffalo heifers on day-7 post oestrus. In conclusion, pretreatment of buffalo heifers with melatonin provides a useful approach to increase the superovulatory response, increases serum total lipids, glucose and reduces the incidence of chromosomal abnormalities and SCE during superovulation. Ultrasound scanning of the ovaries is more accurate in predicting superovulatory response than rectal palpation in superovulated buffalo heifers.

Key words: Buffalo heifers, melatonin, superovulation, ultrasonography.

P9 **DIFFERENT EFFECT OF MELATONIN ON DAILY LH SECRETION IN INTACT AND OVARIECTOMIZED EWES.**

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Increased sexual activity in ewes, which are the short-day breeds, coincides with enhanced secretion of melatonin from the pineal gland. In the last decade evidences were provided that action of melatonin on reproductive function takes place at the level of the hypothalamus and a subset of GnRH neurons responsible for episodic LH secretion in ewes is located within the medial basal hypothalamus (MBH). However, for the activation of the GnRH/LH axis, a several-week long period of the exposition to melatonin is required. The change in estradiol feedback takes place during this time. The aim of the present study was to find out whether and how brief administration of melatonin, directly into the central nervous system, affects daily LH secretion in sexually active ewes and in ewes after ovariectomy (OVX), done during a shift-phase from anestrus to the breeding season. Four ewes, being in luteal phase were infused intracerebroventricularly (icv.) twice: one with the vehicle and once with melatonin, 100µg/100µl/h (total 400µg), in the next cycle. Six OVX ewes were infused icv. three times at weekly intervals: first with the vehicle and the next two times with melatonin, without and after estradiol treatment (OVX+E_2). All infusions were performed afternoon, from 14.00 to 18.00 and blood samples were collected for 4 hours before, during and after the infusions. Statistical analysis of the data in control ewes revealed a significant ($P<0.05$) increase in LH concentration at the beginning of the dark phase, only at 20.30h. The icv. infusion of melatonin did not influenced significantly the basal LH concentration by itself, however, a significant ($P<0.05$) increase in the concentration was noted at 15.00h, one hour from the beginning of melatonin treatment. The secretion of LH in control OVX ewes oscillated around the level which was 3-fold higher than that affirmed in the intact with no significant changes during sampling. A significant ($P<0.05$) decrease in the concentration occurred in OVX ewes within the first 3 hours from the beginning of melatonin infusion. Also in OVX+E_2 ewes, plasma LH concentration was significantly ($P<0.05$) lower during and after infusion of melatonin than that noted before melatonin administration, however, a significant ($P<0.05$) increase in the secretion occurred at 15.00h, one hour from the beginning of the infusion.

In conclusion, melatonin may exert modulatory effect on daily LH secretion in ewes during the breeding season, stimulating the release of this gonadotropin in the presence of estradiol feedback and inhibiting it in case of this steroid deprivation. Thus, estradiol seems to be positively linked with action of melatonin on reproductive activity in ewes.

Key words: melatonin, LH, estradiol.

P10 PLASMA TESTOSTERONE LEVEL OF PUREBRED NATIVE EGYPTIAN BULLS WITH EMPHASIS ON VARIATIONS DUE TO BULL, EJACULATION AND SEASON OF THE YEAR, AND CORRELATION WITH SEXUAL BEHAVIOR AND SEMEN CHARACTERISTICS. Ahmed, W. M. ; Mohamed, A. A. and Abou Ahmed, M. M. * *Departments of Animal Reproduction & A. I., National Research Center and. Theriogenology, Faculty of Veterinary Medicine, Cairo University*, Cairo, Egypt*

This investigation throws a light on the plasma testosterone level in pure breed native Egyptian cattle since few studies were carried out on reproduction in this breed. The study was performed on 6 mature native Egyptian bulls raised in the National Research Center Experimental Farm nearby Cairo. Blood samples were collected at the middle of each month, two samples were collected before semen collection (before 2 and 1 hours) and five samples were collected after semen collection (with ½ hour interval). A single semen ejaculate was collected from each bull once weekly using A.V. Sexual behavior was evaluated in term of the reaction time and numbers of mounts/successful ejaculate. Semen samples were examined and results were correlated with testosterone levels. Testosterone levels were assayed by RIA and data were statistically analyzed. Results revealed that plasma testosterone level of Egyptian bulls averaged 4.07 ±0.43 ng/ml. Differences in plasma testosterone levels due to bulls were highly ($P< 0.001$) significant. Time of semen collection markedly ($P<0.001$) affected plasma testosterone levels. The highest level was found 1 hour before semen collection while the lowest level was noticed 2 hours after semen collection. Season had significant effect on the level at the time of semen collection ($P < 0.001$) as well as 1 hr ($P< 0.001$), ½ and 1½ ($P< 0.01$) and 2 hr ($P < 0.05$) after semen collection. Bull X season revealed highly significant ($P< 0.001$) interaction especially 1 hrs after semen collection. Highly ($P <0.001$). significant interaction was recorded due to bull X season X time variations. The most pronounced correlations were recorded between testosterone levels on one hand and semen volume, individual motility, sperm cell concentration, and initial fructose and citric acid concentrations on the other hand. In conclusion, native Egyptian bulls have a comparable blood plasma testosterone level to the universal breeds with obvious individual, monthly and seasonal variations.

Key words: testosterone, bulls, ejaculation, season, behaviour, semen.

P11 PAPILLARY CARCINOMA OF THYROID OF SHE CAMELS (*CAMELUS DROMEDARIUS*), FIRST RECORD. Youssef F.Ahmed and Hassan M.Desouky. *Dept.Animal Reproduction & A.I.National Research Center, Giza. Egypt*

Thyroid glands of 86 she Camels, aged more than ten years were collected from slaughterhouse at Jeddah, Saudi Arabia during one yeara nd examined for pathological lesions. Gross and histopathological findings revealed that 17cases (19.8%) were diagnosed as cystic follicular hyperplasia (nodular colloid goiter), 3 cases (3.4%) were diagnosed as papillary carcinoma, and the remaining cases appeared within the normal limit. Macroscopically, papillary carcinoma was characterized by uni and/or bilateral enlargement of thyroid lobes associated with presence of multiple nodular projections.On cross section, cystic formation and pale solid scattered areas were seen. The nuclei of anaplastic cells were greatly enlarged in size and vesicular in shape with prominent pseudointranuclear inclusion bodies in some individual cells. This case is considered the first record of occurrence of papillary carcinoma in Camels. Attention should be directed to study the physiopathological changes of endocrine system of Camel (*Camelus dromedarius*).

Key words: Papillary Carcinoma, She camels, Thyroid.

P12 CHARACTERIZATION AND GENE MAPPING OF PORCINE PITUITARY TRANSCRIPTION FACTORS INVOLVED IN GROWTH AND REPRODUCTIVE FITNESS. T. Smith*, K. Sloop, A. Showalter, G. Rohrer*, S. Fahrenkrug*, and S. Rhodes. *Biology, Indiana University – Purdue University Indianapolis, 723 West Michigan Street, Indianapolis IN 46202, USA, and *USDA/ARS U.S. Meat Animal Research Center, Clay Center, NE 68933, USA.*

The mammalian anterior pituitary develops from a derivative of oral ectoderm, Rathke's Pouch, into a mature structure containing five differentiated cell types that are characterized by the hormones that they secrete. These hormones regulate growth, lactation, reproduction, metabolism, and the stress response. Pituitary organogenesis is controlled by the actions of specific transcription factors such as Pit-1, Prophet-of-Pit-1 (Prop-1), Pitx1, Pitx2, Hesx1, SF1, Lhx3, and Lhx4. To begin to characterize the molecular mechanisms that direct pituitary development and function in swine, we have cloned complementary and genomic DNA clones encoding porcine Lhx3 (pLhx3) and Prop-1 (pProp-1). We also have mapped the locations of the *pLhx3*, *pProp-1*, and *steroidogenic factor-1* (*pSF-1*) genes. The DNA binding and gene activation properties of the pLhx3 and pProp-1 factors were analyzed using electrophoretic mobility shift analysis

and transfection protocols. The ontogeny of *pLhx3* and *pProp-1* expression during porcine embryogenesis was analyzed by RT-PCR. The chromosomal location of the *pProp-1* gene was mapped by following the inheritance pattern of polymorphisms located within noncoding regions of the gene. Multipoint analysis determined *pProp-1* to be positioned at 77 cM of the porcine chromosome 1 linkage group (http://www.marc.usda.gov). Using a similar approach, the *pSF-1* and *pLhx3* genes were mapped to 123 and 155 cM, respectively, of the porcine chromosome 1 linkage group, placing both genes within the confidence interval for a known quantitative trait locus (QTL) affecting growth and reproductive traits in swine. Present studies are investigating the role of the *pSF-1* and *pLhx3* genes, with the long-term goal of understanding the molecular basis of genetic features that confer growth and reproductive traits in swine. Supported by the USDA.

Key Words: Growth, Transcription, QTL, Reproduction.

P13 CHARACTERIZATION OF PERIPHERAL IGF-I IN DAIRY COWS SPONTANEOUSLY DEVELOPING OVARIAN CYSTS POSTPARTUM. V.C. Zulu, Y. Sawamukai, T. Nakao*, K. Nakada, Y. Tanaka, M. Moriyoshi. *School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Hokkaido, Japan, and *Graduate School for International Development and Cooperation, Hiroshima University, Kagamiyama 1-5-1, Hiroshima 739-8529, Japan.*

IGF-I is important for follicular development (1), and may therefore play a role in the development of ovarian cysts. This study aimed at characterizing peripheral IGF-I concentration patterns in cows developing ovarian cysts spontaneously postpartum. Five Holstein-Friesian cows, which developed cysts within 60 days, and 5, cycling within 30 days postpartum were used for the study. Ovarian cysts were defined as follicular structures ≥2.5cm present on one or both ovaries in the absence of a corpus luteum. Initial diagnosis was done by palpation per rectum and confirmed by ultrasound. Serum samples were collected once per week, during the dry period and twice per week, 1-2 weeks prepartum for IGF-I, and for 60 days postpartum, 2-3 times per week for IGF-I, progesterone and estradiol determination by RIA. Three and 2 cows developed cysts that persisted (functionally present >25 days, no ovulation) and spontaneously regressed (ovulation of new follicle without treatment), respectively. In all cows systemic IGF-I progressively reduced during the dry period and was lowest at the time of parturition. Immediately postpartum IGF-I levels remained low, rising only after the formation of cysts. Serum IGF-I was significantly higher ($p<0.05$) in cows cycling normally before ovulation (calving to first ovulation) than in cystic cows during cyst formation (calving to diagnosis of cysts) but lower than during the cystic stages (functional existence of ovarian cysts). During the cystic stages, in the 3 cows in which cysts persisted, the IGF-I concentration increased further with the persistence of the cysts and was significantly higher ($p<0.05$) than during cyst formation. During the cystic stages, in the 2 cows in which cysts regressed, the IGF-I concentration was significantly higher ($p<0.05$) than during cyst formation but significantly lower than in the cows in which the cysts persisted. In the cystic cows, serum progesterone concentration fluctuated below 1ng/ml before and during the cystic stages. Serum estradiol was correlated with IGF-I during formation and cystic stages ($r = 0.39 - 0.71$, $p<0.1$). Low systemic IGF-I concentration immediately postpartum may contribute to events leading to anovulation and development of cysts, through a lack of the pre-ovulatory LH surge and LH receptors on granulosa cells of pre-ovulatory follicles. Cysts that spontaneously regress (non-functional) early postpartum, may be characterized by low and those that persist (functional) by high IGF-I concentrations. This study illustrated that a temporal relationship may exist between serum IGF-I and development and functional status of spontaneous ovarian cysts postpartum in dairy cattle.

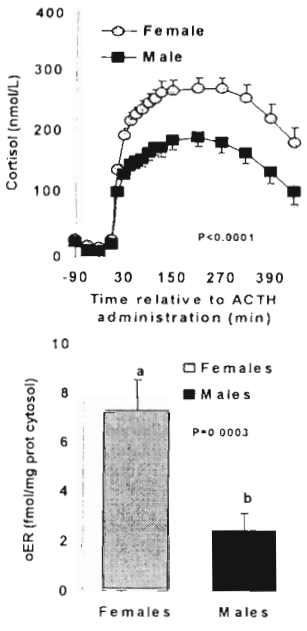
References: (1) Lucy, 2000, *J. Dairy Sci.* **83**, 1635.

Key Words: Bovine, Cystic ovaries, IGF-I

P14 GENDER DIFFERENCES IN ACTH-INDUCED CORTISOL SECRETION AND ADRENAL OESTROGEN RECEPTOR CONTENT IN SHEEP. E. van Lier, S. Åkerberg*, A. Meikle**, R. Pérez-Clariget, M. Forsberg***, L. Sahlin*. *Faculty of Agronomy and **Faculty of Veterinary Medicine, Universidad de la República, Montevideo, Uruguay, *Dept. of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden, ***Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, 750 07 Uppsala, Sweden.*

Sex differences in the response to stress have been described in rodents, with female rats reacting more robustly than males [1], but such data is scarce in sheep. In this study we investigated if ACTH-induced cortisol (C) secretion in sheep is affected by sex and gonadal status and furthermore, the oestrogen receptor (ER) content of the adrenal glands of these animals was determined as an index of oestrogen responsiveness. The study was conducted in late summer

(ACTH administration) and autumn (slaughter). Intact and castrated female (n=15) and male (n=15) Corriedale sheep were used. For the ACTH trial blood samples were taken every 15 min for 9 h. The animals received 0.5 mg of ACTH (Synacthen Depot®, Ciba Geigy, Switzerland) im, after the sample at 1:30 h. C concentrations were determined by RIA. In the autumn all of the sheep of the ACTH trial and 3 more ewes were slaughtered and the adrenal glands were collected, weighed and frozen in liquid nitrogen until determination of ER by ligand binding assay. The MIXED procedure of SAS was used for analysis of variance, with significance at the P<0.05 level. Before ACTH administration, C levels were low and thereafter they were significantly higher in all animals (P<0.0001) until the end of the experiment (see upper panel of figure). Female sheep secreted more C after ACTH administration than male sheep (P<0.0001). The maximum ER levels were registered in the adult castrated ewes (data not shown). The females had higher ER levels than the males (P=0.0003, see lower panel of figure), which could be an indication of the participation of oestrogen in the regulation of C secretion directly at the adrenal gland. This study further supports the existence of gender differences in the hypothalamus-pituitary-adrenal axis of sheep [2].



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Key Words: Adrenal, Cortisol, ER, Sheep, Gender Differences, Male, Female.

P15 EFFECT OF ENVIRONMENTAL HEAT STRESS ON FERTILITY AFTER FIXED TIME A.I. AND ON METABOLIC HORMONES IN DAIRY COW[a]

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In this study, the fertility of post-partum dairy cows after a sequence of treatments with GnRH (day 0), PGF2alpha (day 7), GnRH (day 9) (GPG group; n=82) or hCG (day 0), PGF2alpha (day 7), hCG (day 9) (group HPH; n=83) was investigated in summer and winter seasons. All cows were artificially inseminated 16-18h after end of treatment. Control cows (n = 113) were not treated (CONT Group). Body condition score and milk yields were not significantly different between seasons, number of days post-partum, number of AI's and method of treatment. There were differences (P <0.001) in rectal temperature between hot and cold season (39.2 C° vs 38.5 C°). Within 90 days of calving, the pregnancy rate in CONT cows was lower in summer months compared to the winter months (33% vs 46% respectively) but this effect was not observed in the GPG and HPH groups (37% *vs* 38% for GPG and 40% *vs* 41% for HPH). Comparing treatments, there were differences in pregnancy rates in the hot months (33% for CONT, 37% for GPG and 40% for HPH) but not in cold months (46% for CONT, 38% for GPG and 41% for HPH). The number of days from calving to conception was significantly lower (P<0.001) in GPG and HPH compared CONT cows in cold months (102±3.2, 106±4.2, 126±3.1, respectively; P <0.001) and in hot months (112 ± 3.2, 114 ± 4.2, 139±3.1, respectively; P <0.001). The concentration of IGF-I increased linearly with time post-partum and was not affected by treatment. The concentration of IGF-I showed a significant interaction with time post-partum and season. At calving the concentrations were low and not different from each other. Following calving the concentrations of IGF-I were very low and increased linearly to a maximum at day-70 post-partum (P < 0.001) and the rate of increase was more rapid in winter than in summer (Season X time post-partum interaction P < 0.001). The concentration of IGF-I was not affected by season (P=0.190) or treatment (P=0.260). The concentration of insulin was significantly higher in winter (P < 0.001) and tended to increase with time post-partum (P = 0.077), it was not affected by treatment (P=0.193). There was a significant interaction between season and time post-partum (P=0.044). Glucose tended to be inversely related to the concentration of insulin but they were not significantly different in summer compared to winter (P=0.474). There was no effect of treatment of time post-partum (P=0.110) on glucose concentrations but there was an effect of treatment (P=0.020). There were no significant effects of season (P=0.315) or treatment (P =0.192) on GH concentrations. The concentrations of GH tended to increase with time post partum (=0.055) and the component was significantly increased linearly with time post-partum (P=0.040). In conclusion this study demonstrated that fixed time AI is able to increase pregnancy rate in dairy cows in the hot but not in cold seasons and to decrease the interval from calving to conception all times of the year but with a more evident effect in summer.

Key Words: GnRH, PGF2alpha, hCG, GH.

P16 CYTOGENETIC STUDIES OF *IN VITRO* MATURED AND NON-FERTILIZED/CLEAVED BUFFALO OOCYTES . A.M.Hamam* ; Karima.Gh.M.Mahmoud * M.F. Nawito* ;; A. A.M Seida** and S.M.A. Nawar** . *Dept .Animal Reproduction and A.I, National Research Center, Dokki* ; Faculty of Veterinary Medicine, Cairo University**, Egypt.*

The *in vitro* fertilization of the oocytes represents one of the most important applicable branches that could help in the development of the buffaloes genome . One of the important problems facing the researchers in buffaloe *in vitro* fertilization is the low fertilization rate (21%) as Jainudeen *et al.*(1993). The *in vitro* fertilization now offers the opportunity to study the chromosome abnormalities in gametes.The diploid oocyte has been found to be a more frequent abnormality observed in *in vitro* matured oocytes (Lechniak *et al.*, 1996) .Culture conditions of *in vitro* matured oocytes such as addition of hormones (Kruip *et al.*, 1988) and the reducing temperature of incubation (Aman and Parks , 1994) can affect the meiotic division . The chromosome analysis may illustrate the failure of *in vitro* fertilization (IVF) which are detected through the cytogenetic investigation of the human unfertilized oocytes (Ma *et al.*,1989). Studies about chromosomal abnormalities of unfertilized oocytes were reported in human but little from the available literature was found for animals (Mahmoud K.Gh.M.,2001). Time sequence of meiotic division and accurate chromosome counting of oocytes matured *in vitro* was firstly described in bovine (Ectors *et al.*, 1995 and Sosnowski *et al.*,1996) and buffaloes (Datta and Goswani . 1999 and Mahmoud K.Gh.M , 2001). The present work aimed to :1)evaluate the *in vitro* maturation progress in order to optimize the fertilization rate in buffaloes and 2) to investigate the cytogenetic patterns of oocytes failed to fertilized/cleaved *in vitro* . In a series of experiments , a total number of 1154 oocytes were collected from the ovaries of slaughtered buffaloes, cultured in TCM-199 supplemented with 10% FCS for 21 to 28 hrs at 39°C, 5%Co_2 and 95% relative humidity and examined cytogenetically at this stage . In another trials, matured oocytes were fertilized in vitro by using thawed frozen semen, capacitated in BO medium containing caffeine and heparin for 5 hrs in Co2 incubator. After insemination , the oocytes were co-cultured in TCM-199 for 48 hrs, under conditioned environment. 504 unfertilized/or uncleaved eggs ,which remain undivided in the culture medium after 48 hrs of insemination were subjected to cytogenetic analysis .The oocytes were treated with hypotonic solution, fixed in acetic alcohol fixative and stained with 1% aceto-orcin stain .The meiotic chromosomes were evaluated. The results revealed that the cytogenetic analysis of *in vitro* matured oocytes demonstrated the wide range of the resulting meiotic configurations namely metaphase I(MI), anaphase I(AI), telophase I(TI) and metaphase II(MII).The abnormalities in metaphase II obtained were 5.33%.The percentage of matured oocytes (TI+MII) varied with different culture periods, it averaged 79.36, 83.79, 85.26, 85.71, 88.68, 89.28% and 88.33% at 21,22,23,24,25,26, 27 and 28 hrs respectively .Moreover, the percentage of diploid oocytes was 7.5, 6.67, 3.45, 4.1, 7.14 , 4.54 , 6.25 and 3.85 at culture period of 21, 22, 23, 24, 25, 26, 27 and 28 hrs, respectively. Out of the 346 fixed oocytes, successful chromosome analysis was carried out on 192 oocytes, 136 (70.83%) had a normal haploid complement, 27 (14.06%) oocytes were hypohaploid, 21 (10.94%) oocytes were hyperhaploid and 8 (4.17%) diploid. It is concluded that the maturation rate affected significantly by the incubation period and the suitable time for *in vitro* fertilization of buffalo oocytes is after 24-27 hrs incubation and the chromosomal abnormalities may be responsible in part for low maturation and fertilization rates of buffalo oocytes *in vitro*.

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Key words :Cytogenetic – IVM & IVF- Buffalo oocytes

P17 EFFECT OF MATURATION MEDIA SUPPLEMENTED WITH SERA AND HORMONES ON MATURATION, FERTILIZATION AND EARLY EMBRYONIC DEVELOPMENT OF SHEEP OOCYTES IN VITRO . A.M.Hamam*; Y.R.M.El-baghday**; K.H.El-Shahat** and .M. Mansour**. *Dep. of Animal Reproduction and A.I., National Research Center, Dokki* and Dep. of Theriogenology, Faculty of Veterinary Medicine ,Cairo University** , Egypt.*

In-vitro fertilization of ovulated oocytes in sheep goes back to 1950s when Dauzier and Thibault (1959) and Thibault and Dauzier (1961) presented reports of their work in France. The recent production of lambs (Slavik, et al., 1992 and Holm , et al.,1994) , kids (Younis et al., 1991 ; Nowashari and Holtz (1995) and calves (Brackett, et al.,1982 ; Crister et al. (1986) and Xu, et al. (1987) from oocytes matured and fertilized *in vitro* confirms the fact that these processes can be successfully completed outside the female genital tract. The use of ovaries collected from slaughtered animals at the

abattoir as a source of oocytes for IVM/IVF allows for the large-scale production of embryos that can be used in the development of new biotechnologies such as cloning, sexing and genetic engineering. The development of IVF technology in species such as sheep and goat hold out the promise of useful advances in certain animal biotechnolgy programs such as those involving the production of transgenic animals than would be possible in cattle and livestock with longer generation intervals (Martino et al., 1995). Moreover meiotic maturation occurs spontaneously after removal of the oocyte from the follicles, but can be manipulated by granulosa cell supplementation and media additives (Sirard 1990; Sirard and Bilodeau 1996). However , the efficiency of producing embryos or offspring with *in vitro* techniques is still less than achieved with natural procedures due to the sequential multiplicative effects of a series of seemingly efficient steps in the process of maturation and fertilization in vitro (First and Parrish,1987). The present study aimed to evaluate different maturation media enriched with different sources of sera on maturation and fertilization of ovine oocyte *in vitro*. The ovaries were collected at slaughterhouse, transferred in PBS at 25-30°C.The follicular oocytes (2-4 mm in diameter) were aspirated from ovarian surface using M-PBS and classified into three categories. Class A and B was selected for *in vitro* maturation. Selected immature oocytes were cultured in two different culture media (Ham's F-10 and TCM-199), supplemented with 10% sera (FCS, ESS and LS) BSA, sheep follicular fluid (SFF) and hormones (FSH, LH and estradiol). Antibiotic-antimycotics were added to all solutions and media used.The ovine oocytes were cultured for 24-26 hrs at 38.5°C, 5% Co_2 and high humidity (95%) in Co_2 incubator. After maturation (as indicated by expansion of cumulus cell layers and/or presence of polar body), the matured oocytes were inseminated *in vitro* using ram semen ($2x10^6$ sperms/ml) capacitated in BO medium containing caffeine and heparin for 5 hrs in Co_2 incubator. Inseminated oocytes were co-cultured in maturation medium for 7 days under conditioned environment. The results indicated that higher maturation rates of ovine oocytes were obtained when they were cultured in either TCM-199 or Ham's F-10 enriched with 10% of FCS, ESS or LS than those cultured in the same media enriched with 3% BSA or 10% SFF. A significantly ($p<0.05$) higher fertilization and cleavage rates were explored among oocytes previously matured in TCM-199 enriched with 10% FCS, LS, ESS or SFF than those cultured in the same medium enriched with 3% BSA. However, suitable effects were achieved in Ham's F-10 enriched with 10% FCS, ESS, SFF or LS on the fertilization and cleavage rates of ovine oocytes, but subsequent embryonic development was not appreciably increased. On the other hand, the proportions of morulae and blstocysts were obviously higher on using TCM-199 enriched with 10% LS followed with 10% ESS than 10% FCS and 10% SFF .Maturation, fertilization, cleavage and early embryonic development rates of oocytes matured in TCM-199 medium in combination with 10% FCS and hormones were significantly higher (0.05) as compared with the hormone-free medium

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Key words :IVM – IVF- embryos – sheep –culture media

P18 EXPRESSION OF FSH RECEPTOR AND AROMATASE IN GRANULOSA CELLS OF BOVINE FETAL FOLLICLES.

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Follicular development is dependent on stimulation by growth factors and FSH in the cow (1). Messenger RNA for FSH receptor (FSHr) and Cytochrome P450 aromatase (arom) are expressed in granulosa cells of secondary follicles (2F), early antral follicles (EAF) and vesicular follicles (VF) in bovine ovaries during the estrous cycle (2). However, bovine fetal follicular development and the expression of FSHr and arom in follicles is not well known. The aim of this study was to investigate relationships among follicular appearance, change in the number of follicles and fetal serum FSH concentration, and expression of FSHr and arom in the female bovine fetus. Fetal blood and ovaries were collected from thirty-five Holstein-Friesian female fetuses between 75 and 285 days of fetal age as estimated from the crown-rump length. Sections of the fetal ovary were immunohistologically examined for expression of FSHr and arom in follicles. First appearance of primordial follicles (0F), primary follicles (1F), 2F, EAF and VF was observed in the fetal ovary at day 75, 90, 120, 150 and 240, respectively. The number of EAF increased from 180 days. On the basis of follicular appearance and increase in the number of follicles, fetal age during gestation was divided into 6 stages: 1st stage (day 75-90), 2nd stage (day 90-120), 3rd stage (day 120-150), 4th stage (day 150-180), 5th stage (day 180-240), 6th stage (day 240-285). Serum FSH concentration in the fetus increased significantly at 4th and 5th stages than 1st, 2nd and 3rd stages. Expression of FSHr was not observed in pre-granulosa cells of 0F throughout gestation, but was observed in

granulosa cells of 1F at 3rd, 4th and 5th stage, and in granulosa cells of 2F and EAF at 3rd, 4th, 5th and 6th stages. Expresseion of FSHr corresponded with expression of arom throughout all stages. These results indicated that FSHr and arom in granulosa cells of 1F and 2F were expressed at 3rd stage before the increase in fetal FSH. Appearance of EAF and increase in the number of EAF corresponded with the remarkable increase of fetal FSH and expression of FSHr and arom in granulosa cells of 2F and EAF. Thus, development from 1F to 2F may be related to expression of FSHr and arom in the fetal ovary, and development from 2F to EAF may be related to increase of FSH and expression of FSHr and arom in the fetal ovary.

References: (1) McNatty et al., 1999, *J. Reprod. Fertil. Suppl.* **54**, 3; (2) Bao et al., 1998, *J. Anim. Sci.* **76**, 1903

Key Words: Bovine, Fetus, FSH, Aromatase.

P19 ***IN VITRO*** **SECRETIONS OF STEROIDS, PROSTAGLANDINS AND PROTEINS BY PORCINE TROPHOBLAST COLLECTED DURING IMPLANTATION.** B. Szafranska*, G. Panasiewicz*, M. Woloszyn*, J. Klos**, A.J. Ziecik** **Dept. Animal Physiology, Faculty of Biology, University of Warmia and Mazury, 10-718 Olsztyn, **Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, Poland.*

A successful implantation depends on the establishment of appropriate secretions of porcine conceptuses (embryos and trophoblast). The objective of our study was to determine production of steroids, prostaglandins (PG) and porcine pregnancy-associated glycoprotein (pPAG) family [1] secreted *in vitro* by trophoblast recovered from gilts during time of implantation (Days 15-16) and its cultured explants for 52 days. Secretory proteins (>10 kDa) were separated from media and analysed by SDS-PAGE (2-10 μg/sample), then pPAG proteins were detected by Western blotting with polyclonal antisera. Separated small fractions (<10 kDa) were lyophilised and subjected to concentration measurements of estradiol-17β (E2), estron (E1), testosterone (T), androstenedione (A4) performed by RIA with previously produced antisera. Prostaglandin E2 (PGE2) and F2α metabolite (PGFM) were estimated by ELISA. Steroids were measured with lately characterised anti-E2 sera [2] and various validated anti-steroids sera. Cross-reactivity of used anti-E1 sera was <0.04% with dehydroizoandrosterone, 5α-androstane 17β-ol-3-one and <0.01% with several other steroids. Cross-reactivity of anti-T sera was 55.5% with 5α-androstan-17β-ol-3-one, 8.36% with 4-androstan-11β-ol-3,17-dione, 0.47% with 5β-androstane-3α-17β-diol and <0.09 with others. Cross-reactivity of anti-A4 sera was 57.8% with 5α-androstane-3,17-dione, 22.46% with epiandrosterone, 13.9% with 5β-androstane-3α-17β-dione, 9.41% with androsterone, 4.57% with testosterone and <0.2% with others. Results showed secretory activity of porcine trophoblast *in vitro*. Western blotting indicated ~43 kDa native pPAG proteins. Total steroidogenic activity of trophoblast explants (1.35±0.33 g) throughout 1238 hrs *in vitro* was (x±SEM): 217.9±13.9 pg of E1, 1 053.9±49.2 pg of E2, 368.0±15.2 pg of T and 533.3±102.2 pg of A4. Steroids were synthesised with various efficiency: 18.4±3.7 of E1, 88.8±15.9 of E2, 30.4±4.8 of T and 45.3±12.6 of A4 pg/100 mg of trophoblast explants. Total PG production was 3 145.5±1 524.4 ng of PGE2, 11.3±9.25 ng of PGFM with their secretion rate: 200.4±57.45 and 1.04±0.87 ng/100 mg of trophoblast explants, respectively. The production efficiency *in vitro*, calculated per mass of explants, revealed high secretory activity of porcine trophoblast. Thus, appropriate secretion/interaction between pPAG proteins and E1, T, A4, PGE2 may be involved in embryonic mortality during conceptus attachment, implantation and can modulate protective role of E2 during early pregnancy maintenance in the pig (*Grants: KBN-PO6D-011-13 and UWM 020600.804).

References: 1 Szafranska *et al.*, 1995, *Biol. Reprod.* **53**, 21; 2 Szafranska and Tilton, 1993, *J. Reprod. Fertil.* **98**, 643

Key Words: E1, E2, T, A4, PGE2, PGFM, pPAG, implantation

P20 **PLEMENTARY DNA (cDNA) OF pPAG3, A NOVEL MEMBER OF PORCINE PREGNANCY-ASSOCIATED GLYCOPROTEIN 1-LIKE GENE SUBFAMILY (pPAG1-LIKE).** G. Panasiewicz, B. Szafranska. *Department of Animal Physiology, Faculty of Biology, University of Warmia and Mazury, 10-718 Olsztyn-Kortowo, Poland (szafran@uwm.edu.pl)*

Distinct cDNA of PAG genes have been cloned in ungulate species: *Artiodactyla*, *Perissodactyla*, *Carnivora* and *Rodentia* [1-3]. The first previously identified cDNA of porcine PAG genes, pPAG1 and pPAG2 (GenBank: L34360 and L34361) represent two subfamilies that code chorionic proteins structurally related to aspartic proteinases, i.e. pepsins, cathepsins D and E [1]. Lately, next two cloned and sequenced cDNAs of pPAG4 and pPAG6 (GenBank: AF272734 and AF272735, respectively) were assigned to the pPAG2-like subfamily, in which at least 8 genes are expected. But, a total number of pPAG1-like genes remain still unknown. The objective of our study was to characterise the pPAG1-like gene subfamily expressed in pre-placenta (trophoblast). A porcine conceptus cDNA library (Day 13-17) was screened with ^{32}P-labeled PAG probe to identify novel cDNAs. Obtained positive phages were plaque-purified

then converted into phagemids from Lambda ZAP II by *in vivo* excision procedures. Among 7 isolated cDNAs, 5 were similar to pPAG2 and 2 were similar to pPAG1. The longest cDNA of selected clone (#103C) was arbitrarily named pPAG3 and finally sequenced in both directions by the standard dideoxy-nucleotide procedure. The 5'-untranslated region (UTR), the open reading frame and 3'-UTR of pPAG3 cDNA were 11, 1170 and 176 bp, respectively. A comparison of pPAG3 to pPAG1 indicated high nucleotide sequence homology (99.4%) and amino acid (aa) identity (98.4%) of polypeptide precursors containing 389 aa. Identified mutations in nucleotide sequence of pPAG3 coded several aa substitutions: N→K^{36}, R→S^{113}, LK→FR$^{226-227}$, R→Q^{234}, R→A^{371} (numbering after 15 aa signal peptide removal during post-translational precursor processing). The comparison of residues surrounding aspartic acids (D) within a catalytic cleft of the pPAG2-like subfamily (^{77}VFDTGSS^{83} and ^{260}IVDTGTS^{266}, according to pepsins numbering) showed aa substitutions in pPAG3 (IFDTASS and ILDSGSA). Moreover, pPAG3 precursor contained two aa insert (QA$^{283-284}$, coded by CAGGCC), various than in porcine pepsin (GA), pPAG4 and pPAG6 (NA), within a hypervariable loop domain ($^{281-287}$ aa) and located on the surface of all PAGs. The polypeptide sequence of the pPAG3 revealed 4 potential sites of N-glycosylation (N-x-S/T), at positions 64, 104, 115 and 333 (processed precursor numbering). In conclusion, our study reports novel pPAG3 cDNA deposited in the GenBank database Acc. No.. AF315377 (Supported by KBN grant PO6D-011-13).

References: [1] Szafranska *et al.*, 1995, *Biol. Reprod.* 53, 21; [2] Xie *et al.*, 1997, *PNAS* 94, 12816; [3] Green *et al.*, 1999, *Biol. Reprod.* 60, 1069

Key Words: cDNA, pPAG3, pPAG1-like gene subfamily, pig

P21 **PREGNANCY-ASSOCIATED GLYCOPROTEINS SECRETED *IN VITRO* BY CHORIONIC EXPLANTS OF WILD (*Bison bonasus L.*) AND DOMESTIC UNGULATES.**

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Pregnancy-associated glycoproteins (PAG) are encoded by several distinct genes expressed in extraembryonic cells (trophoblast) during implantation and in chorionic epithelium (trophectoderm) of ungulate species possessing different type of placentas [1-3]. Distinct complementary DNA (cDNA) of the PAG family have been cloned in different animals: hoofed *Artiodactyla* (pig, cattle, sheep, goat) and *Perissodactyla* (horse, zebra), or non-hoofed species, *Carnivora* (cat) and *Rodentia* (mouse). These PAG cDNAs have been isolated from cDNA libraries or were amplified by reverse transcription (RT-PCR), then cloned and sequenced. Multiple PAG-like genes were also identified by Southern analysis of genomic DNA isolated from leukocytes of several wild animals. Some of identified PAG cDNAs code native PAG proteins that were purified from placenta of various ruminants (cattle, zebu, sheep, goats, elk, and moose) then used as RIA standards for diagnostic tests of early pregnancy. The purpose of our study was to determine and compare a profile of the PAG proteins secreted *in vitro* by chorion of wild and domestic animals. Extraembryonic membranes (trophoblast / trophectoderm) were recovered from early pregnant cows or gilts at a local slaughterhouse and cotyledons were collected *post mortem* from eliminated European bison. Trophoblast explants collected from pigs (Days 16, 17, 18, 19, 20) were cultured for 384 up to 768 hrs. Cotyledons and trophectoderm, collected from ruminants (Days 35-150), were cultured for 72 hrs. Total proteins were isolated from media. Separated crude proteins (>10 kDa) were analysed (10μg/sample) by SDS-PAGE and PAG-like proteins were detected by Western blotting with polyclonal anti-PAG sera produced against native proteins or antisera raised against recombinant protein pPAG2. Western analysis indicated various M_r of PAG proteins during long-term *in vitro* studies. A major ~45 kDa and smaller quantity of ~78, ~67, ~65 and ~30 kDa pPAG-like proteins were secreted by cotyledons of European bison. A major ~73 kDa and ~67, ~65, ~45, ~30 kDa PAG proteins were secreted by bovine cotyledons, and 43-72 kDa by porcine trophoblast explants. Equal quantity of similar in M_r PAG proteins were also produced by extra-cotyledons explants of bovine trophectoderm. This is the first paper indicating the immunodetection of the PAG proteins produced *in vitro* by placental explants of European bison. Such productions of placental proteins can be useful for isolation of native PAG proteins required as RIA standards for tests of early pregnancy diagnosis (*Supported by KBN grant PO6D-011-13 and UWM 020600.804 to B.Sz.).

References:[1]Szafranska *et al.*1995, *Biol.Reprod.***53**,21; [2]Xie *et al.*1997,*PNAS*,**94**,12816; [3]Xie *et al.*1997, *Biol.Reprod.***57**,1384.

Key Words: European bison, chorionic proteins, PAG, pregnancy diagnosis.

P22 SECRETION OF INHIBINS IN THE MALE GOTTINGEN MINIATURE PIGS. W Jin [†], KY. Arai [†], C B. Herath[†], M Kondo [†], H Ishi[‡], Y Tanioka[‡], G Watanabe [†], N P. Groome and K Taya [†] *Department of Basic Veterinary Science, The United Graduate School of Veterinary Science, Gifu University, Gifu 501-1193, Japan; [†]Laboratory of Veterinary Physiology and [‡] Department of Tissue Physiology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan; [‡]Central Institute for Experimental Animals, Kanagawa 213-0001, Japan, School of Biological and Molecular Science, Oxford Brookes University, Oxford, UK*

The expression of inhibin subunits in the testes of the Gottingen miniature pig was examined by *in situ* hybridization and immunohistochemistry. In addition, the circulating and testicular inhibin A, inhibin B and inhibin pro-C were measured by enzyme-linked immunosorbent assays (ELISA). Positive immunostaining for inhibin B subunit was observed in Sertoli cells, Leydig cells, and late stage germ cells. Leydig cells, Sertoli cells and spermatogonia were stained for inhibin A subunit. On the other hands, positive immunostaining for the inhibin B subunit was observed in the Leydig cells and spermatogonia. In contrast to inhibin C and A subunits staining specificity, inhibin B did not positively stained in the Sertoli cells. The *in situ* hybridization revealed that although the inhibin B subunit mRNA signal was highly expressed in all cell types. Inhibin A subunit mRNA expression was somewhat identical to that of the inhibin B subunit and germ cells appeared weakly stained. Strong positive mRNA signal for inhibin B subunit was confined to the Leydig cells and late stage germ cells but not in Sertoli cells. ELISA results showed that concentrations of inhibin B and inhibin pro-C were high in both the circulation and the testis. In contrast, inhibin A levels in both plasma and testes were undetectable. The present results strongly suggest that inhibin B is the major form of circulating inhibins and that the Leydig cells are the predominant source of this dimeric inhibin in male Gottingen miniature pigs. Furthermore, the germ cells also appear to be an important source of circulating inhibins.
Key Words: testes, inhibin, Sertoli cell, Leydig cell, mRNA

P23 THE EFFECT OF INFUSING GLUCOSE OR GLUCOSAMINE OR OF FEEDING A LUPIN GRAIN SUPPLEMENT ON THE NUMBER OF FOLLICLES AND AROMATASE GENE EXPRESSION IN OVARIES FROM GnRH TREATED ANOESTROUS SHEEP. M. Muñoz-Gutiérrez1,3, G.B. Martin2, D. Blanche2 and R.J. Scaramuzzi1. *1Dept of Veterinary Basic Sciences, Royal Veterinary College, London NW1 0TU, UK, 2Dept of Animal Science, University of Western Australia, Nedlands WA, Australia. 3Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana Iztapalapa, Mexico City, Mexico.*

Improved nutrition increases ovulation rate in sheep and published evidence suggests that this response is mediated by intra-ovarian energy-pathways (1-4). An experiment was conducted to compare the effect of energy sources on folliculogenesis. Nineteen anoestrus Merino ewes were fed with a diet of only straw hay (Control, n=5) or supplemented with lupin grain (500g/day, n=4) or infusions of glucose (50 mmol/h, n=5) or glucosamine (3.5 mmol/h, n=5). Intravaginal progestagen sponges were inserted for 12 days and nutritional treatments started 5 days before their removal and continued to the end of the experiment. At sponge removal the ewes were given a series of GnRH injections (2.5 mg every 4 h from 0 to 8 h followed by 2.5 mg every 2h from 10 to 16 h and then 1.25?mg every hour from 17 to 30 h) to induce follicular development. Thirty hours after sponge removal, the ewes were euthanised and their ovaries collected and stored at -70o C. The ovaries were serially sectioned (10 mm), every 20th section was stained with haematoxylin and eosin (to count follicle number and to measure follicle diameter) and each 17th to 18th section was tested by in situ hybridisation for aromatase using an homologous oligonucleotide probe. Data were analysed by ANOVA. There were effects of treatment on the number of follicles <1mm ($P = 0.018$); 1-2 mm ($P = 0.078$); >2-3 mm ($P = 0.057$) and >3-4 mm ($P = 0.049$).Thirty-four aromatase-positive follicles were detected in ovaries from seventeen ewes spread across all 4 groups (diameter range 1.57 to 7.93mm). In ten animals, the largest follicle was aromatase-positive. The mean diameter of the aromatase-positive follicles was largest in lupin fed group and smallest in the glucosamine infused group (Mean ± sem; 4.25 ± 1.91, 3.88 ± 0.21, 5.25 ± 1.39 and 3.51 ± 0.94 for control, glucose, lupin and glucosamine, groups respectively; $P = 0.012$). However, there were no differences in jugular venous concentrations of FSH, or LH suggesting that the action of increased energy supply on follicular growth and the induction of aromatase gene expression is a direct effect on the developing follicles.
References: 1) Nottle et al., 1997, Anim. Reprod. Sci. 49, 29; 2) Rhind et al., 1998, Anim. Reprod. Sci. 52, 131; 3) Boukhliq et al., 1996, Reprod. Sci. 45, 59; 4) Downing et al., 1997, Theriogenology. 47, 747.
Key words: Follicular development, Aromatase, Ovary, Nutrition, Sheep.

P24 **PREDICTION OF FIRST LACTATION YIELD FROM A PRE-PUBERTAL GH SECRETAGOGUE CHALLENGE IN DAIRY HEIFERS.** V.J. Taylor*, D.E. Beever**, M.J. Bryant**, D.C. Wathes*. *Reproduction & Development Group, Royal Veterinary College, EN6 1NB, UK. **Centre for Dairy Research (CEDAR), The University of Reading, RG6 6AT, UK

This study investigated the potential of metabolic hormone profiles in pre-pubertal female dairy calves to act as a predictor of first lactation milk yield. Previous studies have found that the release of growth hormone (GH) following a secretagogue challenge was related to genetic merit in dairy calves[1]. Methods: 50 Holstein-Friesian heifer dairy calves (5-7 months old) of average or high pedigree index (PI) were cannulated in the jugular vein the day prior to a 10 hour serial bleed session. Calves were challenged once with bovine growth hormone-releasing factor (bGRF)(1-44) (0.2 microgrammes/kg live body weight). Following puberty the same heifers were served by AI (mean age at conception, 430 d). Thirty-eight of these heifers were subsequently monitored throughout their first lactation. They were managed indoors post-calving and fed a total mixed ration (TMR) *ad libitum*. Calangates were used to monitor dry matter intake (DMI) and the heifers were milked three times per day. Results: A mean peak GH concentration of 99 ± 10.8 ng/ml was achieved by 10 minutes post-challenge in the calves. A GH challenge response variable was calculated (mean of +5, +10, +15 and +20 min post-challenge GH divided by mean of -15 and –5 min pre-challenge GH). Data were log transformed for statistical analyses. Animals (mean PI value: 61 ± 1.7; mean 305d milk yield: 7417 ± 191.2 kg) were divided into average (AP, 40-60, n=15) and high (HP, 61-86, n=23) PI groups; then low (<7400 kg) or high (>7400 kg) 305d milk yield groups to give: AP, low yield (APLY, n=8); AP, high yield (APHY, n=7); HP, low yield (HPLY, n=9) and HP, high yield (HPHY, n=14). AP calves that subsequently produced a high yield (APHY) had a greater (P=0.09) GH peak response than those that produced a low yield (APLY). A significant (R=0.59, P<0.05) positive correlation between GH challenge response and first lactation 305 day milk yield in average PI heifers was established. In contrast there was no difference in GH response to GRF between high PI heifers producing high or low yields and in these animals there was a negative relationship between the GH response and 305 day yields. Whereas first lactation milk yield performance was reflected by pre-pubertal GH challenge response in average PI animals, these data suggest that other factors may have limited yield in the high PI animals in their first lactation. The GH-IGF-I axis is involved in both lactation and reproduction. GH secretagogue challenges may also reveal differences between dairy animals likely to be fertile or develop reproductive problems and warrant further investigation.

References: (1) Løvendahl *et al.*, 1991, *Can. J. Anim. Sci.* **71**: 1045.

Key words: dairy heifers, GH, GRF, lactation

P25 **ESTIMATION OF RELEASING OF PROSTAGLANDINS F_2 ALFA ($PGF_2\alpha$) DURING THE PRE AND POSTPARTUM PERIOD .** S.G Hassan[1] , K.A. El-Battawy[2]; A.A. El-Menoufy[3]; M. Younis[4] and R.M. Khattab[5] *National research Centre, Dokki, Guiza, Fact Vet. Med. Cairo Univ., Anim. Prod. Institute, Minist. Agric.*

This study was carried out on ten late pregnant buffalo five days prepartum and one week postpartum. The postpartum female buffalo makes a series of physiological readjustments in both the uterus and ovaries to restore her reproductive capacity (1). Several studies (2,3) concluded that the luteolytic release of $PGF_2\alpha$ will terminate the life span of CL during the short cycle postpartum. They recorded in cows that I.M. injection of 25 ml $PGF_2\alpha$ twice daily for 10 days starting on day three postpartum achieved complete uterine involution about 7-10 days earlier than the control Hence the aim of this work was to estimate the values fo $PGF_2\alpha$ before and after parturition. Material and Methods: 1. Blood samples were collected from all animals five days before parturition and 7 days postpartum; 2- Determination of $PGF_2\alpha$ by ELISA (4). Results and discussion : It is observed form the present work that the level of prostaglandin $F_2\alpha$ on day 3, 2 and 1 prepartum was higher than on day 5 prepartum (91.30 ±2.52, 96.85 ± 1.58, 101.10 ±0.99 and 81.45 ± 4.32 pg/ml respectively). A sudden shap increase in the $PGF_2\alpha$ conc. (180.83 ± 4.23 pg/ml) occurred on the day of delivery (day O) followed by a gradual decrease on the plasma concentration of $PGF_2\alpha$ on day 4, 5, 6 and 7 postpartum (67.93 ± 4.28, 62.61 ± 2.86, 50.1 ± 3.07 and 41.30 ± 2.17 pg/ml respectively). Our values determined here are in agreement with those reported in buffaloes (5) who recorded that the level of PGF_2± was low then increased gradually till the day of parturition where it achieved a peak of 213.50 pg/ml.

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Key words : PGF2α, pre- and postpartum, cows

P26 ENDOGENOUS PROGESTERONE PROFILE IN GOATS IN SEASONAL ANESTRUS AT LATITUDE 20°28'S. *A.C.D.Monreal,** G.H.Toniollo, **Area of Animal Breeding. "Universidade Estadual Paulista" - Unesp -Botucatu/SP - Brazil. Rua Teodomiro Carmelo,371-18609-580, Botucatu/SP – Brazil, **Area of Animal Breeding e Obstetrics. Universidade Estadual Paulista - Unesp - Jaboticabal/SP - Brazil*

The profile of endogenous progesterone in seasonal anestrous goats was studied at the latitude 20°28'S to verify if there is or not ovarian activity at this time of the year. Mestizo goats (29) were submitted to the natural photoperiod from May to December 1997. Progesterone in plasma was dosed by RIA. These animals were monitored daily and paired twice a day in an attempt of observing the ruts along the experiment. A group of goats under artificial photoperiod (control) (26) was submitted to the same treatment of blood sampling and it was kept 250 meters away from the research group. The beginning of the ruts appeared after 08 weeks from the end of the artificial photoperiod. Only one animal of the study group just manifested the estrus (3,4%), it was covered but did not conceive. All the animals of the control group (artificial photoperiod) manifested estrus (100%) and they were covered by the respective male goats on the property (05). The fertility (0%) and the prolificacy (0) obtained in the studied group was compared to the control group (69,2%) (1, 6), respectively. For the two variables it was significant ($p< 0,01$) with (= 5% for the control group). In spite of just one goat having manifested the estrus, it didn't show a level of plasma progesterone compatible with the estrus, however, 09 goats of the study group (11,11%) presented ovarian activity, by the plasmatic progesterone dose in spite of not having manifested estrus. The seasonal anestrus is present in this latitude and some animals presented ovarian activity without manifestation of the estrus, with individual character and varying from 0 to 9,47 ng/ml. The hormonal profile of the study group was low in relation to the group of the artificial photoperiod.
References: Jaramillo et al., 2001; Nlassoued et al.,1995; .Parraguez et al., 1994
Key words: goats, ovarian activity, (P4) progesterone

P27 THE EFFECT OF FEEDING A DIET TO INCREASE CIRCULATING INSULIN CONCENTRATIONS ON REPRODUCTIVE PERFORMANCE IN DAIRY COWS. J.G. Gong, K.D. Troup, E. McCullough, P.C. Garnsworthy*, R. Webb*, D.G. Armstrong. *Roslin Institute (Edinburgh), Roslin, Midlothian EH25 9PS, UK and *School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK.*

We have previously shown that the first ovulation after calving is delayed in dairy cows selected for high milk yield, associated with a lower circulating insulin concentration. Furthermore, feeding a diet to increase peripheral insulin concentrations during the first 50 days postpartum can advance the first ovulation. This study extended the duration of dietary treatment to 100 days to examine the effects on reproductive performance. The experiment was a 2 by 2 factorial design, involving high (h) and low (l) genetic merit and high (H) and low (L) starch diet (n=10 per group). The 2 diets were balanced in energy and protein content and fed as complete rations as in normal farm practice. During the first 50 days plasma samples were collected 3 times a week for insulin assay. Starting from the first day of milking, whole milk samples (20 ml) were collected 3 times a week for 105 days and assayed for progesterone by RIA. Farm records were kept of service dates and pregnancy diagnosis outcome, daily milk yield, and weekly body weight (BW) and body condition score (BCS). Whilst the cows from high line produced more milk and lost more BW and BCS during the experiment, no significant effects of the diet were observed on these measurements. Plasma insulin concentrations were 0.39±0.016, 0.28±0.012, 0.30±0.013 and 0.21±0.010 ng/ml in the lH, lL, hH and hL groups respectively. Both effects of genetic merit and diet were significant ($p<0.01$). Conception rates to the first service were 50.0, 20.0, 33.3 and 12.5 % in the lH, lL, hH and hL groups respectively. The effect of diet was significant ($p<0.05$). There were also effects of the diet on other fertility measurements including interval from calving to the first service, interval from calving to conception and number of service required per conception, as derived from farm records and milk progesterone profiles. The genetic merit of cows also had a negative effect on fertility measurements, but no significant interactions between diet and genetic merit were observed. This study has confirmed our previous observations that reproductive performance is reduced in high genetic merit cows. Moreover, the results have shown that the diet can improve fertility without negatively affecting milk yield and energy balance status, suggesting that nutritional management may provide a viable approach to alleviate the fertility problems in high yielding dairy cows.
(Supported by MAFF).
Key words: Insulin, Nutrition, Fertility, Dairy cattle.

P28 AN INHIBITOR OF NITRIC OXIDE SYNTHASE (L-NAME) BLOCKS PROSTAGLANDIN $F_{2\alpha}$-INDUCED LUTEOLYSIS IN CATTLE. D.J. Skarzynski, J.J Jaroszewski*, M.M. Bah, K.M. Deptula, B. Barszczewska*, B. Gawronska. *Institute of Animal Reproduction and Food Research, Tuwima-St 10, Olsztyn 10-747, Poland; Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-719 Olsztyn, Poland**

Although it has been well documented *in vivo* that luteolysis is brought about by prostaglandin (PG) $F_{2\alpha}$, it does not inhibit basal progesterone (P4) secretion by either small and large bovine luteal cells *in vitro* (1). This discrepancy had led us and other (1) to postulate that a number of substances produced locally in bovine CL may mediate the luteolytic action of $PGF_{2\alpha}$. Recently we shown that administration of an inhibitor of NOS (L-NAME) in late of the cycle increases P4 secretion and prolongs the functional life of the bovine CL (2). Moreover, the increased effects of $PGF_{2\alpha}$ on luteal cells pre-exposed to NO suggest that NO priming of bovine CL is needed to complete the regression of bovine CL (3). Therefore, we determined whether NO may mediate $PGF_{2\alpha}$-induced regression of the bovine CL *in vivo*. Mature holstein heifers (n=12; Day 15 of the estrous cycle) were used in this study. One day before the experiment, a polyvinyl chloride catheter was inserted into aorta abdominalis through the coccygeal artery. The tip of canniula was positioned cranial to the origin of the ovarian artery to allow for the direct infusion of drugs to the reproductive tract. In Control Group the heifers (n=4) were infused for 60 min with 20 ml of Saline and at 30 minute of the infusion, 2 ml of Saline were injected (i.a.). Next four heifers were infused with Saline and injected with analogue of $PGF_{2\alpha}$ (aPGF; Cloprostenol; 100 μg). The last group of animals was infused with L-NAME (400 mg) and injected with aPGF. Cloprostenol given at time of Saline infusion, shortened (17.8±0.4 d) the cycle duration compare with that of the Control (21.8±0.3 d). Although aPGF temporarily increased P4 output, the P4 concentration was decreased ($P<0.05$) after 60 min of treatment. L-NAME increased P4 secretion and extended luteotropic action of $PGF_{2\alpha}$ ($P<0.05$). The luteolytic action of aPGF was blocked by L-NAME, as shown by the cycle duration (22.4±0.3 d). Finally, we established that NO is produced in bovine CL. We investigated changes in NADPH-diaphorase (NADPH-d) activity (marker for NO synthase) and the distribution of inducible (i)NOS and endothelial (e)NOS proteins by immunohistochemistry in bovine CL during the estrous cycle. NADPH-d activity was present in bovine CL with the highest activity at mid- and late luteal stages. eNOS was observed with the strongest immunostaining in the late CL. Also iNOS was localized in bovine CL with the highest intensity at the late luteal phase. These results, along with our pervious demonstrations (2,3), suggest that NO plays an important role in the regulation of luteal regression in cattle mediating the luteolytic action of $PGF_{2\alpha}$ on the CL.

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Key Words: Luteolysis, nitric oxide, $PGF_{2\alpha}$, Corpus luteum

P29 TUMOR NECROSIS FACTOR-α REGULATES PROSTAGLANDIN $F_{2\alpha}$ AND E_2 SECRETION FROM BOVINE OVIDUCT: POSSIBLE ROLE OF NITRIC OXIDE. K.M. Deptula, M.M. Bah, K. Okuda*, D.J. Skarzynski. *Institute of Animal Reproduction and Food Research, Tuwima-St 10, Olsztyn 10-747, Poland; Faculty of Agriculture, Okayama University, 700-8530 Okayama, Japan**

The oviduct plays a critical role in reproduction by being the site of the gamete maturation and transport, fertilization and early embryonic development. It has been shown that the secretory and motor activities of the oviduct are regulated by several ovarian and uterine factors. However, the auto/paracrine mechanisms of the locally produced prostaglandins (PGs) in the bovine oviduct are controversial (1). Recently, we found that tumor necrosis factor-α (TNFα), acting partially by nitric oxide (NO), stimulates PGs secretion in bovine endometrium (2,3). Since TNFα mRNA and TNFα protein have been detected in embryo of several species, TNFα may play a role in the regulation of the PGs and NO production in the bovine oviduct. The present study was undertaken to determine this supposition. Bovine oviducts were classified into two stages (estrus: Day 0; postovulatory phase: Days 2-4). After 1 h of pre-incubation, oviductal explants from the ampulla or isthmus (20-30 mg) were incubated in the absence or presence of TNFα (0.6 nM), S-NAP (a NO donor; 10 μM) and AMT (a competitive inhibitor of inducible isoform of NO synthase; 10 μM) for 6 h. Although TNF α and S-NAP stimulated $PGF_{2\alpha}$ output from ampulla ($P<0.001$), the isthmus was only feebly sensitive to any treatments at both examined stages ($P\geq0.05$). The action of TNF-α and NO donor on $PGF_{2\alpha}$ output was two-times stronger at the postovulatory phase than at the estrus ($P<0.05$). Additionally, the basal production of PGF_2 in ampullary segments was two-times higher than in isthmus of the oviduct ($P<0.01$). In contrast to $PGF_{2\alpha}$ secretion, TNF α- and NO-stimulated PGE_2 output was higher in the isthmus than in ampulla of the oviduct ($P<0.01$) and differed at examined phases of the cycle. Moreover, TNFα stimulated production of nitrite/nitrate (NO_2/NO_3; stable NO metabolites) in the bovine oviduct as shown by measurement of NO_2/NO_3 concentration in the conditioned medium

using the SIGMA calorimetric method. In contrast to TNFα actions, AMT inhibited output of both PGs, i.e., $PGF_{2\alpha}$ in ampulla and PGE_2 in isthmus of the oviduct ($P<0.05$). Moreover, the inhibition of the activity of inducible NOS strongly reduced production of NO_2/NO_3 in the bovine oviduct at both examined phases of the cycle. These results indicate that TNFα is a potent regulator/modulator of $PGF_{2\alpha}$ and PGE_2 secretion in the bovine oviduct. NO may be involved in this process. These findings suggest that there are specific properties in the bovine oviduct, dependent on the stage of the cycle and on the region of oviduct, to regulate of PGs production. Further studies are in progress to determine the physiological significance of these findings in terms of the regulation of bovine oviductal motility at the time of gamete transport and early embryo development.

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Key Words: TNFα, Nitric oxide, Prostaglandins, Oviduct

P30 EFFECTS OF EARLY MATERNAL DEPRIVATION ON THE NEUROENDOCRINE RESPONSES OF YOUNG RABBITS. G. Brecchia, D. Zampini, G. Guelfi, F. Mazza*, A Bonanno*, C. Boiti. *Dipartimento di Scienze Biopatologiche veterinarie, University of Perugia, I-06100 Perugia, Italy, *Istituto di Zootecnica, Faculty of Agronomy, University of Palermo, Palermo, Italy.*

Temporary dam-litter separation (DLS) technique has been shown to increase fertility of lactating does up to 20% (1, 2). Although viability of young rabbits is not affected after 48 h of maternal deprivation, the question whether this kind of stress applied in the early days of life exerts on them any long-term and lasting effects remains unanswered. This question was addressed by evaluating their reproductive performance and the responses of the hypothalamic-pituitary-adrenal (HPA) axis in both young and mature female rabbits. To this end, at parturition (day 0), hybrid lactating does of same parity (24/group), age (8-9 months), weight (3.5-3.8 kg) and litter size (8-9) were randomly assigned to control or DLS group. Control does had free access to the nest for nursing, whereas DLS does were separated from their litters from day-9 to day-11 after parturition. Acute stress was evoked on 14-days old rabbits of both groups by means of saline injection and a 5-min confinement in a closed box. Afterward, each young rabbit returned to its nest box until sampling 5, 15 and 30 min later. Blood samples (one/each animal) were collected by cardiac puncture within 30 sec after removal from the nest box. To another set of 120-days old rabbits, belonging to both control and DLS groups, stress was induced by ACTH (30 μg/kg, Synacthen-Depot, Novartis) or saline injection. Blood samples were collected via catheter of the marginal ear vein 0, 30, 60, 90 and 120 min after treatment. All the blood samples were immediately centrifuged and plasma stored frozen until assayed for corticosterone by RIA (CORT kit ICN, Biochemicals). In a parallel experiment carried out on a commercial rabbit farm, a pre selection of 71-days old females belonging to both control and DLS groups was done based on health condition and body weight (>1.8 kg). After the first screening of 150 young does, the final selection of 125 females was done before AI, when they aged 115-days, using the same criteria and body weight (>3 kg). AI was performed according to a 42-day reproduction rhythm, using two batches, without any hormonal stimulation besides GnRH for induction of ovulation. At day 14, control rabbits displayed much lower ($P<0.05$) basal plasma corticosterone concentrations than maternal deprived rabbits (0.2 *vs.* 2 μg/dl, respectively), but enhanced reactivity to acute stress as assessed by a 50-fold raise 30 min later (10 vs 2 μg/dl, respectively). At day 120, plasma levels of corticosterone in response to ACTH were long lasting and comparable in both groups over the 120 min period examined. By contrast, after saline injection corticosterone levels were higher ($P<0.05$) in control than in DLS rabbits. Fertility rate at first insemination was higher in maternal deprived than in control rabbits (72.8±6.3 vs. 63.9±6.1) as well as number of total born (7.4±0.48 vs. 6.7±0.50), and born-alive (6.0±0.52 vs. 5.3±0.55). In conclusion, our data suggest that 2-days-long maternal deprivation in young rabbits influences differently their neuroendocrine responses of the adrenal as well as ovarian axis.

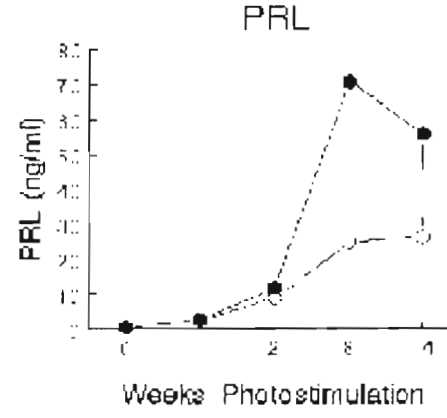
References: Theau-Clèment, 2000, *7th World Rabbit Congress* **1**, 61; Bonanno et al., 2000, *World Rabbit Sci.* **7**, 171.

Key Words: Maternal deprivation, Rabbit, Corticosterone, Fertility

P31 EFFECTS OF PROGESTINS AND OESTROGENS ON TRANSCRIPTIONAL ACTIVITY OF THE CYTOCHROME-$P450_{scc}$ GENE ($P450_{scc}$) IN A STABLE PORCINE GRANULOSA CELL LINE *IN VITRO*. M.C. Agostini, P.M. Bartlewski, M.A. Furlan, A.D. Smida, P.J. Chedrese. *Dept. Obstetrics, Gynaecology & Reproductive Sci., Reprod. Biol. Res. Unit, College of Medicine; University of Saskatchewan, Saskatoon, SK, Canada.*

From earlier studies mainly performed in rodents, it is plausible that locally produced steroids exert autocrine, paracrine and endocrine effects on ovarian steroidogenesis. Similar information for other species is limited. It has been shown that

oestradiol-17β (E_2), progesterone (P_4) and the synthetic progestogen levonorgestrel (LNG), increase progesterone accumulation in the stable porcine granulosa cell line, JC 410 (1). The mechanisms whereby steroid hormones stimulate progesterone synthesis in the granulosa cells are not fully understood. The objective of this study was to examine the effects of P_4, LNG, E_2 and the synthetic oestrogen 17α-ethinyl oestradiol (EE; 0.1, 1, 10, 30 and 100 μM) on transcriptional activity of the cytochrome-$P450_{scc}$ gene ($P450_{scc}$), transiently expressed in the JC 410 cells. The relative transcription rates of $P450_{scc}$ were determined by a luminometric assay, following the transfection of 2320 bp of $P450_{scc}$ linked to the luciferase (LUC) gene. 0.1, 1 and 10 μM P_4 increased (*P<0.05*) the transcription of $P450_{scc}$ by 1.7-, 1.8- and 2.0-fold, respectively, whereas 30 μM had no effect (0.9-fold; *P>0.05*) and 100 μM completely suppressed transcription (0.03-fold; *P<0.001*). LNG, over the entire range of concentrations studied, did not affect (*P>0.05*) the transcription of $P450_{scc}$. E_2, at 10 and 30 μM, significantly increased the rate of transcription of $P450_{scc}$, by 1.4- and 1.7-fold, respectively, with stimulation reaching a plateau at 100 μM (1.6-fold). Treatment with EE resulted in 1.5-, 1.6-, 2.4-, 2.8- and 2.5-fold increases (*P<0.05*) in transcriptional activity of $P450_{scc}$, respectively. The present results suggest that ovarian progestins and oestrogens can both regulate the transcription of $P450_{scc}$ in the porcine JC 410 granulosa cells, in a gonadotrophin-independent manner (JC 410 cells do not respond to gonadotrophins, hence the effects of steroids seen were independent of gonadotrophic influences). Low concentrations of P_4 and high concentrations of E_2 increased, whereas high concentrations of P_4 decreased the transcription of $P450_{scc}$. In addition, LNG appeared to be considerably less and EE more potent than P_4 and E_2, respectively. Lastly, as LNG stimulated progesterone secretion in the JC 410 cells (1), its local effects on steroidogenesis cannot be explained solely by the control of $P450_{scc}$ expression.



Reference: (1) Rodway *et al.*, 1999, *J. Ster Biochem. Mol. Biol.* **68**, 173-80. Funded by NSERC and Saskatchewan Health/CIHR grants to P.J.C.

Key words: Progestins, Oestrogens, Cytochrome-$P450_{scc}$, Pig Granulosa Cells

P32 RELATIVE AND ABSOLUTE PHOTOREFRACTORINESS IN TURKEY HENS: PROFILES OF PROLACTIN, THYROXINE, AND TRIIODOTHYRONINE EARLY IN THE REPRODUCTIVE SEASON. J. A. Proudman, *USDA, ARS, GGPL, Beltsville, MD 20705 USA and T. D. Siopes, Dept. of Poultry Science., North Carolina State Univ., Raleigh, NC 27695 USA.*

Reproduction in turkeys is controlled by photoperiod and is a balance between two physiological states, photosensitive and photorefractory (PR). The hen requires a period of short day lengths to establish photosensitivity and then photostimulation (PS) is thought to both initiate egg laying and program the onset of the PR response by the presence of thyroxine (T4) during the early weeks following PS. The turkey is considered an absolutely PR species, meaning that when gonadal regression occurs in the presence of a stimulatory photoperiod the hen cannot resume laying without first being exposed to short days. Interestingly, all turkeys do not express PR during their first lay cycle and this provides a useful model for the study of PR. The present experiment was designed to determine 1) whether plasma levels of prolactin (PRL), T4, or triiodothyronine (T3) early in reproduction may be associated with presence or absence of PR later in the reproductive season; and 2) whether a relative PR response occurs in turkey hens. Turkey hens were stimulated with a 18L:6D photoperiod and blood samples were collected prior to PS and at 1, 2, 8 and 14 wk after PS. At 19 wk after PS, half the hens were transferred to 13L:11D, also a stimulatory photoperiod, for 4 weeks. Hens which ceased lay (considered PR) were returned to 18L for an additional 4 wk. Hens which then resumed egg laying were considered relatively PR, while those that did not were considered absolutely PR. Hormone profiles comparing hens which became absolutely PR with hens that lay throughout the 27 weeks PS showed markedly lower PRL levels at 8 and 14 wk PS in hens that subsequently became PR. Of hens exposed only to 18L, 20% became absolutely PR. Thus, a substantial portion (about 80%) of our flock of commercial-strain turkey hens never exhibited PR throughout a 27 wk reproductive season. Of layers exposed to 13L, 30% ceased lay (considered PR) within 4 wk. Of those hens returned to 18L from 13L, 50% resumed lay and thus were considered relatively PR. These results are consistent with both relative and absolute PR responses occurring in turkey hens. In addition, hormone profiles early after PS indicated differences between PR and non-PR hens for prolactin but not for thyroid hormones.

Keywords: photorefractoriness; hormones; turkeys.

P33 CHARACTERIZATION OF A STEROIDOGENIC CAPRINE LUTEAL CELL LINE IMMORTALIZED BY A TEMPERATURE-SENSITIVE SIMIAN VIRUS 40. C.H Chiu, I.C. Guo*, C.L. Chen**, J.H. Lin, L.S. Wu. *Department of Animal Science, *Department of Veterinary Medicine, National Taiwan University, Taipei, **Depr Occupational safety and Health, China Medical College, Taichung, Taiwan, Republic of China.*

An ideal *in vitro* model for the study of endocrine functions would consist of cells that are able to proliferate in culture and express specialized functions (1). A temperature-sensitive luteal cell line from rat has been established, but the function of progesterone secretion didn't expressed (2). The purpose of this study was to establish and characterize a progesterone-synthesizing caprine luteal cell line, named tsCLC-D. Luteal cells were obtained from goat during the middle stage of estrous cycle. The primary cells (CLC) were cultured at 37° in Medium 199. CLC were then infected with a temperature-sensitive mutant strain of simian virus 40 and cultured at 34° in 5 % fetal calf serum-Medium199 until transformation. Cloned cells were isolated and cultured at 34° and tested at both 34 and 40°. Vehicle, oLH, cAMP, 22-hydroxycholesterol and pregnenolone were added to wells at the time of temperature shift. The progesterone concentrations of media were determined by an enzyme immunoassay. Cells following different treatments were harvested for Western blot analysis of large T antigen and steroidogenic enzymes. The results showed that the tsCLC-D was temperature-sensitive for morphology, cell proliferation, and progesterone production. At the permissive temperature (34°), these cells were spindle-shaped and grew rapidly. However, at the nonpermissive temperature (40°), the cells exhibited a rounded shape, and had a good response to 22-hydroxycholesterol and pregnenolone treatments but not to oLH and cAMP additions. These treatments increased progesterone biosynthesis and steroidogenic enzyme expression of cells. The level of expression of 3β-HSD and P450scc enzyme in tsCLC-D is lower than that of in primary luteal cells. We concluded that tsCLC-D might have lost the response to gonadotropins during the process of immortalization, but it still retained the ability of progesterone production. The temperature-sensitive caprine luteal cell line can provide a unique model for the study of regulation of progesterone biosynthesis *in vitro*.

Reference: Chedrese *et al.*, 1998, *J. Mol. Endocrinol.* **20**, 287; Sugino *et al.*, 1998, *Endocrinology* **139(4)**, 1936.

Key Words: Goat, Luteal cells, Cell line, SV40.

P34 SEASONAL EFFECTS OF SUBTROPICAL CLIMATE ON CORTISOL TURNOVER AND ADRENAL RESPONSIVENESS IN TAIWAN NATIVE GOATS. L. S. Wu, C. C. Chou*, J. H. Lin. *Department of Animal Science, National Taiwan University, *Taipei Zoo, Taipei, Taiwan, Republic of China.*

The adrenal cortex, an organ of homeostasis, plays a role in stress adaptation. Under natural conditions, severe seasonal heat exposure results in decrease plasma glucocorticoids in ruminants (1, 2). There is no report of cortisol turnover and adrenal response to adrenocorticotropin (ACTH) in goats. The Taiwan native goat is a rare indigenous breed existing in Taiwan. The goats have the ability to tolerate heat stress in subtropical Taiwan. This study was designed to investigate the effects of natural climatic environment on circadian rhythm in plasma cortisol, the rate of cortisol turnover, and adrenal responsiveness in Taiwan native goats. Experiments were taken in summer (Jul.-Sep., average temp. $29.3_{\pm}1.5$; RH $77.8_{\pm}2.7\%$) and winter (Nov.-Jan., average temp. $20.1_{\pm}2.6$; RH $80.0_{\pm}0.5\%$), respectively. The plasma cortisol concentrations were determined by an enzyme immunoassay. In circadian cortisol rhythm trial, blood samples were collected from jugular cannula at 20 mins interval during a 24-hr period. The results showed that there is a circadian rhythm of plasma cortisol levels. The peak of cortisol level was found at 0900 to 1100 hr in winter and 0300 to 0700 hr in summer. The average concentrations, basal concentrations and frequency of episodes of plasma cortisol in winter were $6.4_{\pm}2.3$ ng/ml, $5.3_{\pm}2.0$ ng/ml and 10.5 pulses/24 hr, respectively. In summer, those values were $4.5_{\pm}1.8$ ng/ml, $3.0_{\pm}0.9$ ng/ml and 12.1 pulses/24 hr, respectively. No significant difference was observed between two seasons. Clearance of cortisol from the plasma of goats was evaluated by two-compartment model. The half-life of cortisol was longer in winter than in summer (40.8 vs. 27.4 min.)($P<0.05$). The secretion rate ($5.3_{\pm}1.8$ vs. $5.2_{\pm}1.2$ ug/min), and metabolic clearance rate ($0.8_{\pm}0.2$ vs. $1.0_{\pm}0.2$ l/min) of cortisol were no significant different between two seasons ($P>0.05$). In ACTH stimulation test, the responsiveness of cortisol to exogenous ACTH was lower significantly in summer than in winter ($P<0.05$). The low plasma cortisol and reduced adrenal response to injection of ACTH probably reflects adaptation to the hot climate and a mechanism to prevent high metabolic heat in goats.

Reference: Wu *et al.*, 1992, *J. Chin. Soc. Anim. Sci.* **21(1)**, 67; Guerrini *et al.*, 1982, *Br. Vet. J.* **138**, 175.

Key Words: Goat, Cortisol, Hot season.

P35 EFFECT OF PRENATAL EXPOSURE TO 2,3,7,8-TCDD ON ONE-DAY OLD CHICK PARAMETERS. V. Bruggeman and E. Decuypere. *Lab of Physiology of Domestic Animals, Faculty of Agricultural and Applied Biological Sciences (K.U.Leuven), Kasteelpark Arenberg 30, B-3001 Leuven (Belgium).*

From literature, it is well known that the most critical and sensitive period for exposure to hormonal active compounds out of the environment and from food is during embryonic/fetal development. In this experiment, the chicken embryo model was used to investigate the possible effects of prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on one-day old chick and liver weights and on thyroid and estrogen plasma concentrations. On day 5 of incubation, 3 doses of TCDD (2, 6 and 18 ng/egg ; 100 µl/egg) were injected into the egg via a small hole in the egg shell at the side of the air chamber. Control eggs were injected with 100 µl oil. After injection, eggs were taped off and incubation was proceeded under normal incubation conditions. At hatch, chicks were weighed, checked for abnormalities and phenotypic sex was determined by feather sexing. All hatched chicks were killed by decapitation, livers were weighed and blood was collected for measuring tri-iodothyronin (T_3), thyroxin (T_4) and estradiol (E_2) by RIA. Mortality was lowest in the 2 ng group (55.3 %) and highest in the 18 ng group (75.7 %). The number of hatched chicks with imperfect navel closure was higher in the chicks exposed to TCDD *in ovo*. Absolute chick and liver weights were not different between controls and TCDD injected chicks ; however, the liver/chick weight ratio tended to increase with increasing dosis of TCDD. T_3 concentrations were lower in the TCDD injected chicks reaching significance in the 2 and 6 ng group ($p<0.05$) while T_4 concentrations showed the inverse pattern with the lowest level in the control group ($p<0.05$). T_3/T_4 ratio was significantly higher in the control group compared to the TCDD injected chicks. No macroscopic changes at the gonadal level were observed. E_2 concentrations significantly differed between sexes but were not affected by TCDD. In conclusion, injection at embryonic day 5 of incubation with 2,3,7,8-TCDD resulted in relative heavier liver weights. T_3/T_4 ratio was significantly lowered by TCDD which could be related with the higher incidence of imperfect navel closure in these groups. This experiment shows that the chick embryo is very sensitive to low concentrations of TCDD with consequences on one-day old chick parameters. The effects of prenatal injection with TCDD on postnatal growth, with special attention to the thyroid axis, needs further investigation.

Key words : 2,3,7,8-tetrachlorodibenzo-p-dioxin, chicken embryo

P36 IMMUNOLOCALIZATION OF AROMATASE IN THE PORCINE GONADS. M. Słomczyńska, M.Kotula-Balak, B.Bilińska. *Laboratory of Animal Endocrinology & Tissue Culture, Institute of Zoology, Jagiellonian University, 30-060 Kraków, Poland*

Aromatization of androgens into estrogens is performed by a microsomal enzyme, the cytochrome P450 aromatase. A direct approach for identifying the cellular source of aromatase is the use of immunohistochemistry with a specific antibody that recognizes aromatase. The pig presents some unusual features with regard to the synthesis of testosterone and estrogens in male and female gonads (1, 2). In testes from prepubertal males, testosterone level measured radioimmunologically, was lower than in testes from adult pig, while estrogen secretion was relatively high and comparable to that of mature porcine gonad. Immunolocalization of aromatase in testes (3) from both immature and mature pigs was confined to the Leydig cell cytoplasm. The intensity of immunohistochemical staining was not the same within the interstitial area, indicating the heterogenous Leydig cell population. Other somatic cells and germ cells were negative for aromatase. In porcine ovaries the expression of aromatase was correlated with the stage of follicular development and state of corpora lutea regression. In early stages, granulosa cells were very weakly stained while aromatase was maximally expressed in the large preovulatory follicles. As porcine theca cells have some aromatising ability, aromatase was localized in those cells. Corpora lutea from the early luteal phase showed positive staining, whereas those from midluteal phase did not stain for aromatase, some cells of regressed corpora lutea unexpectedly exhibited aromatase staining. In control tissue sections incubated with omission of the primary antibody or in the presence of normal rabbit serum, no positive staining was observed. Supported by a grant DS/ZFZ/IZ/2001

References. 1. Conley et al., 1996, Biol. Reprod. 54, 497; 2. Conley et al., 1994, Biol. Reprod. 51, 655; 3. Levallet et al., 1998, Biol Reprod 58, 919

Key Words: aromatase, testes, ovary, pig

P37 **LOCALIZATION OF FSH RECEPTOR IN THE PORCINE OVARY.** M. Słomczyńska, M. Duda, M. Szołtys *Laboratory of Animal Endocrinology & Tissue Culture, Institute of Zoology, Jagiellonian University, 30-060 Kraków, Poland*

The basic functional units of mammalian ovaries are follicles at various stages of their development, and different types of corpora lutea depending on the stage of reproductive cycle. Several aspects of growth, development and steroidogenesis in ovarian follicles are controlled by the interaction of steroid hormones and gonadotropins (1). Follicular production of estrogen is dependent upon both FSH stimulation of aromatase in the granulosa cells and LH stimulation of androstendione production by theca cells. FSH is an obligatory hormone to maintain normal function of the ovary to release oocytes and hormones in all mammalian species. The FSH receptor is found exclusively on granulosa cells as early as in two-layer or primary follicles. We sought to characterize the ovarian expression of mRNA for the FSH receptor as well as resulting protein expression. The mRNA for FSH receptor was detected in serial sections by *in situ* hybridization which provides a powerful tool for studying the cell-specific expression of mRNA in the follicles and corpora lutea (CL) at all stages of their development and regression. During growth and differentiation of follicles, mRNA for FSHR was expressed exclusively in the granulosa cells. The corpora lutea did not express FSHR at any stage of regression. A direct approach for identifying FSHR distribution is the use of immunohistochemistry with a specific antibody that recognizes FSH receptor (a generous gift of Dr J. Dias) (2). The immunostaining with monoclonal anti FSHR antibody revealed the presence of the receptor in the cytoplasm of granulosa cells. No FSHR mRNA was expressed in CL regardless of the stage of regression. It is worth stressing that the dominant follicle becomes increasingly dependent on LH, which in turn stimulates both androgen synthesis and aromatization. Supported by a grant BW/ZFZ/IZ/2001

References. 1. Richards et al., 1995, Rec. Prog. Horm. Res. 50, 223; 2. Peterson et al., 2000, Mol. Cell. Endocrinol.160,203

Key words: FSH receptor, porcine ovaries

P38 **ATRESIA OF LARGE OVARIAN FOLLICLES OF THE RAT.** M. Szołtys, M. Słomczyńska, Z. Tabarowski*, A. Sakiewicz. *Laboratory of Animal Endocrinology & Tissue Culture and *Laboratory of Haematology and Toxicology, Institute of Zoology, Jagiellonian University, 30 060 Cracow, Poland.*

More than 99.9% of the follicles present in the mammalian ovaries undergo degenerative changes called atresia. A cohort of antral follicles which develops at the beginning of pregnancy in the rat can serve as an interesting physiological model of this process. These follicles produce small amount of oestradiol during periimplantation time and then degenerate. The main objective of the present study was to detect apoptotic cells appearing in these follicles, and to analyse changes in localisation of androgen receptors (ARs). Pregnant Wistar female rats were killed in succession until day 7 of pregnancy. Excised ovaries were submitted to a routine histological procedure. Apoptotic cells were detected using the *In situ* cell death detection kit. ARs were localised using an immunohistochemical method. Some sections were stained with H&E. Histological observations revealed that the investigated group of follicles grew slower than their previously investigated cyclic counterparts and reached preovulatory size and morphological appearance on day 5 of pregnancy. However, they did not ovulate and on day 6 and 7 of pregnancy numerous apoptotic cells appeared within these follicles. They were localised predominantly in the antral granulosa layer and were especially numerous near the cumulus oophorus complex (COC) and in the region linking COC with the follicular wall. In this way COC was becoming separated from the follicular wall. AR immunostaining was located predominantly in the nuclei of the granulosa layer of preantral and very early antral follicles, present in the ovaries of pregnant rats. However, the differentiation of investigated antral follicles was accompanied by the centripetal disappearance of AR but until day 5 AR depletion did not include COC. This pattern of AR depletion resembled that typical of follicles maturing during the oestrous cycle, although lasted longer. On day 5 and 6 of pregnancy ARs were still present in some COCs, similarly as in the cyclic preovulatory follicles. However, in some COCs immunoreaction was very weak or they were almost completely devoid of ARs. In the present study the process of atresia presumably resulted from high concentrations of prolactin and progesterone. Prolactin is known to suppress follicular oestradiol production while progesterone could afflict maturing follicles by inhibiting LH release, necessary for intensive oestradiol production and induction of ovulatory processes. Slower AR depletion could reflect the suppression of $P450_{arom}$ expression.

References: Hillier, 1985, Oxford Rev. Reprod. Biol. **7**, 109; Szołtys & Jabłonka, 1989, Fort. Zoolog. **35**, 103; Szołtys & Słomczyńska, 2000, Exp. Clin. Endocrinol. Diabetes **108,** 228.

Key Words: Apoptosis, Androgen Receptors, Ovarian Follicles

P39 NON CONVENTIONAL ENZYMES ARE RESPONSIBLE FOR THE BIOSYNTHESIS OF PROSTAGLANDIN F2 alpha IN BOVINE ENDOMETRIUM IN VIVO AND IN VITRO. Eric Madore, Joe A. Arosh, Pierre Chapdelaine and Michel A. Fortier. *Ontogénie et Reproduction, Centre de Recherche en Biologie de la Reproduction, Centre de Recherche du CHUL and département de gynécologie obstétrique,Universitié Laval,Québec GIV 4G2, Canada.*

In cows, endometrial prostaglandin $F_{2\alpha}$ ($PGF_2\alpha$) is the luteolytic hormone whereas PGE_2 may favor maternal recognition of pregnancy . So far, it has been assumed that COX1-2 and the common PGFsynthases identified in bovine lung and liver were responsible for the production of $PGF_2\alpha$ in endometrium. We have found that COX-2 but not COX-1 mRNA is present and maximal from days 13-21 of the estrous cycle. At the same time, we have found expression of PGEsynthase mRNA at high levels. Amazingly, known bovine PGFS were not detected in endometrial tissues or cultured cells despite of documented and abundant production of $PGF_2\alpha$. Since PGFsynthases belong to the large aldo-keto-reductases (AKR) family, we have sought for related enzymes that could be responsible for the production of PGF_2 . In the present study, total RNA was extracted from endometrial tissues collected on days (1-3), (4-6), (7-9), (10-12), (13-15), (16-18) and (19-21) of the estrous cycle. The expression of AKR-1B and AKR-1C and PGFS (bovine lung & liver type) mRNAs was analyzed by Northern blot. In parallel , RNA was extracted from primary cultures of endometrial epithelial and stromal cells producing high levels of $PGF_2\alpha$ and PGE_2 respectively. Wide spectrum oligo primers specific for the AKR-1C and 1B family were generated using concensus sequences of bovine lung, liver, DDBX and 9K-PGR for 1C and AKR-1,3,5,7 and 9 for 1B respectively. There was no expression of the 1C family at any time of the cycle or in cultured endometrial cells, but strong expression in control lung and liver tissues. Interestingly, the AKR-1B primers detected a strong signal in endometrial tissues between days 13-21 and in cultured endometrial cells. These and our previous results suggest that in the endometrium, specific unknown enzymes able to use a wide spectrum of substrate including PGH_2, PGD_2 and PGE_2 are regulated to control the relative production of PGE_2 and $PGF_2\alpha$ and effect luteolysis or recognition of pregnancy. Supported by NSERC of Canada.
Key words: PGF2α, Endometrium, bovine

P40 OVARIAN FUNCTION AFTER INDUCTION OF OVULATION WITH A DESLORELIN IMPLANT IN NONLACTATING DAIRY COWS. JA Bartolome[1], SM Pancarci[2], LF Archbald[1], and WW Thatcher[2]. [1]*Department of LACS, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA, and* [2]*Department of Animal Sciences, University of Florida, Gainesville, Fl 32611, USA.*

The objective of this study was to compare the effectiveness of two doses (750 and 1000 μg) of a Deslorelin implant with gonadorelin diacetate (GnRH, 100 μg), to induce ovulation and regulate subsequent follicular and CL development. Nonlactating dairy cows (n=20) were injected on d –9 with 100 μg IM GnRH, and with two doses of 25 mg IM PGF_2 8 h apart on d-2. On d 0, cows were assigned randomly to three treatment groups and received either 100 μg IM GnRH (Group 1, n=6), an implant containing 750 μg SQ Deslorelin (Group 2, n=7) or an implant containing 1000 μg SQ Deslorelin (Group 3, n=7). On d 16, cows were injected with PGF_2 (two 25 mg doses 8 h apart) to induce luteolysis. Ovaries were evaluated by ultrasonography daily from d 0 until ovulation and every other day until d 36. Daily blood samples were collect from d 0 until either ovulation after PGF_2 given on d 16 or until d 36 to determine plasma progesterone (P_4) concentrations. The Heat Watch® system was used to monitor estrus. The outcome variables were plasma P_4 concentrations (ng/ml), follicular waves during diestrus (yes/no), follicular development (mm), and expression of estrus after luteolysis (yes/no). All cows ovulated and formed a CL after treatments on d 0. Plasma P_4 concentrations from d 0 to d 16 were not different between cows in Group 1 (5.9 ± 0.6 ng/ml), Group 2 (6.4 ± 0.6 ng/ml), and Group 3 (6.3 ± 0.6 ng/ml). However, plasma P_4 concentrations were higher on d 11 for cows in Group 3 (11.1 ± 1.0 ng/ml) compared to cows in Group 1 (8.2 ± 1.1 ng/ml; $P<0.05$) and on d 12 for cows in Group 2 (12.4 ± 1.0 ng/ml) compared to cows in Group 1 (9.0 ± 1.1 ng/ml; $P<0.05$). The number of cows that showed follicular waves from d 0 to d 16 was greater ($P<0.001$) in Group 1 (6/6) compared to Groups 2 (1/7) and 3 (0/7). The average diameter of the largest follicles from d 16 to d 20 was larger ($P<0.001$) in cows in Group 1 (16.1 ± 0.9 mm) compared to cows in Groups 2 (6.3 ± 0.8 mm) and 3 (4.7 ± 0.8mm). Cows in Group 1 expressed estrus and ovulated a dominant follicle (6/6) within 4 days after PGF_2 on d 16. In contrast, only one cow in Group 2 (1/7) and none in Group 3 (0/7) expressed estrus within 4 days after PGF_2 ($P<0.001$). The rate of follicular growth from d 16 to d 36 was greater in cows in Group 2 (0.67 mm/d) than in cows in Group 3 (0.4 mm/d; $P<0.01$). Twelve of 14 (86 %) cows in Groups 2 and 3 expressed estrus between d 24 to d 36 (1 in estrus on d 19 and 1 did not show estrus) but failed to ovulate. The 12 cows that failed to ovulate underwent an Ovsynch protocol when the largest follicle reached 20 mm in diameter; and 10/12 (83%) ovulated synchronously in response to the second GnRH injection. In conclusion, Deslorelin implants induced ovulation, stimulated development of a normal corpus luteum, and delayed follicular growth during subsequent

diestrus. Utilization of a deslorelin implant as part of a timed insemination protocol to improve embryo survival warrants further investigation, but will require re-synchronization of nonpregnant cows.
Key words: GnRH, ovulation, progesterone, cattle

P41 **IGF-I, IGF-BINDING PROTEINS, GH AND IGF-I RECEPTORS IN HYPOTHYROID AND HYPERTHYROID NEONATAL PIGS**. *I. Louveau*, P. Herpin. *INRA, Unité Mixte de Recherches sur le Veau et le Porc, 35590 Saint Gilles, France.*

Both the GH/IGFs and the thyroid axes are considered to play a major role in the control of growth, development and metabolism. The present study was undertaken to get a better understanding of the interaction between these two axes in the neonatal period. The influence of thyroid status on plasma IGF-I and IGFBP levels and on GH and IGF-I receptor levels in liver and skeletal muscle was examined under conditions of controlled milk intake. Twenty four newborn unsuckled piglets were allotted to one of three groups corresponding to control (C, n=8), hypothyroid (Hypo, n=8) and hyperthyroid (T3, n=8) piglets. Hypothyroidism was induced by *i.p.* injection of methimazole (40 mg/kg BW/d) and iopanoic acid (60 mg/kg BW). In the T3 group, this treatment was completed by the injection of triiodothyronin (T3; 40 µg/kg BW/d) to induce a moderate hyperthyroidism. Saline was administered to C piglets. Animals were bottle-fed sow colostrum then milk formula during the whole experiment. At 7 days of age, piglets were killed and blood was collected to determine plasma IGF-I and T3 concentrations by radioimmunoassay and IGFBP levels by ligand blotting. Liver and skeletal muscle were also sampled to quantify ^{125}I-bovine GH (bGH) and ^{125}I-IGF-I specific binding and receptor mRNA expression by RNase protection assays. Plasma T3 concentrations differed ($P<0.05$) between the 3 groups (C: 2.26 ± 0.21; Hypo: 1.34 ± 0.15; T3: 4.82 ± 1.14 nmol/L). Plasma IGF-I and 29 kDa IGFBP levels were higher in the T3 group ($P<0.05$) than in the other groups. Specific binding of ^{125}I-bGH was higher ($P<0.05$) in the liver of T3 group than in the other groups. It was lower in skeletal muscle (LD; $P<0.05$) of the hypo group compared to the other groups. Specific binding of ^{125}I-IGF-I was higher in skeletal muscle of the T3 group than in the other groups whereas it did not differ between the 3 groups in liver No significant difference was observed for GH and IGF-I receptor mRNA in theses tissues. These findings are consistent with the tissue-specific regulation of both GH and IGF-I receptors and support the existence of an interaction between the thyroid and the GH/IGF-I axes.
Key Words · T3, IGF-I, IGFBP, GH, Receptor, Piglet, Muscle

P42 **LAYING HEN GONADOTROPE RESPONSES TO DIFFERENTIAL PULSE FREQUENCIES OF cGnRH-I *IN VITRO*** . M.C.Soñez*, G. Delhon*, C.A. Soñez**, M.T. Mugnaini**, R. Dezi***, I von Lawzewitsch*. *Área Histología y Embriología, Fac.Cs.Vet. Universidad de Buenos Aires (U.B.A.) Chorroarín 280. (1427) Buenos Aires, Argentina. ** Fac.Agr.-Vet.Universidad Nacional de Río Cuarto (U.N.R.C.), Argentina.*** Fac. Cs.Exactas U.B.A. INEUCI-CONICET, Buenos Aires, Argentina. E-mail: mcsonez@fvet.uba.ar

The GnRH pulse frequency exerts differential effects on FSH-LH gene expression and gonadotrophin secretion in mammals.[1] In avian gonadotropes, cGnRH-I promotes LH secretion *in vivo* and *in vitro*.[2] The aim of this work was to determine hen gonadotrope changes after administration of cGnRH-I 1-10 nM every 15, 30 or 60 min. pulse-frequencies *in vitro*. Re-aggregates of primary adenohypophysial cells cultures of 17 week old laying hens (Brown & Nick) were stimulated on plates using cGnRH-I (Sigma) 1-10 nM in four 5 min-pulse every 15, 30 or 60 min frequencies. Pulse frequencies and dose-dependent effects were observed in six experiments (three wells/plate for each assay). Initial and final controls were included in each experiment. Immunocytochemical method (ABC-Vector Labs.Inc) was used with anti-chFSH (1:6000), anti-chLH (1:8000) as primary antibodies (Dr.J.A.Proudman, USDA-ARS, Maryland, USA). FSH-LH-trope numbers were obtained by using stereological analysis (VIDA-K-system)[3]. Data were statistically processed with ANOVA, Kruskall-Wallis, Scheffe or Bonferroni tests. Gonadotrope groups were defined according to the following parameters: isolated (I), aggregated (A), immunoreactive (+), non immunoreactive (-), and used to determine differences. Significative differences were found for FSH-tropes I (-) at 1 nM dosis 30 vs 60 min pulse frequencies ($p=0.045$; 3.52±0.49% and 1.51±0.24% , SE), A (+) cells did not show statisticant differences at any GnRH doses or pulse frequencies. Differences were found in A (+) LH-tropes at 1 nM cGnRH-I, 15 vs 30 min ($p=0.001$), 30 vs 60 min ($p=0.013$) (4.67±2.32%-16.69±1.63%, 16.69±1.63-7.74±1.67%,SE respectively); A (-) LH-tropes at 1nM cGnRH-I, 15 vs 30 min ($p=0.001$), 30 vs 60 min ($p=0.005$) (90.29±1.52% - 79.97±1.78%, 79.97±1.78% - 88,89±1.53%, SE, respectively); A (+) LH-tropes at 10 nM cGnRH-I, 30 vs 60 min ($p=0.028$), A (-) LH-tropes 30 vs 60 min ($p=0.026$) (14.74±1.28%-7.39±1.32%, 82.91±1.95%-90.66±1.18%, SE, respectively) and I (-) LH-tropes to 10 nM cGnRH-I, 15 vs 30 min ($p=0.04$), 15 vs 60 min ($p=0.018$) (3.7±0.48% - 1.4±0.70%, 3.7±0.48% - 1.37±0.29%, SE,

respectively). These results suggest that LH-trope responses were cGnRH-I dose-dependent at a pulse frequency of 15 min.but not of 60 min, showing changes in A (+) percentages. 1 nM cGnRH-I effectively stimulated LH secretion in aggregated cells. 10 nM cGnRH-I administered in 60 min. pulses were effective in stimulating LH secretion in both closely aggregated and isolated cells at 15 min pulse frecuency. The differences in isolated FSH-tropes at 30 and 60 min pulse-frequencies suggest that, in our system, FSH secretion is probably under the control of the neighboring cells. Supported by grants from the University of Buenos Aires, Argentina (UBACYT- TV-040)

References: (1) Kaiser U.B., *et al,* 1997, Endocrine Rev.**18** (1),46; (2) Chou H. e*t al*, 1985, Life Sci.**37**, 2459; (3) Weibel E.R.,1979, Vol.**1**, Academic Press, London.

Key Words: cGnRH-I, pulse-frequency, hen gonadotropes, primary cultures.

P43 **PLASMA INSULIN, GLUCAGON AND LEPTIN CONCENTRATIONS IN TRANSITION COWS.** T. Kokkonen, S. Alasuutari, A. Tesfa, M. Tuori, H. Manner, L. Syrjälä-Qvist. Department of Animal Science, P.O.Box 28, 00014 University of Helsinki, Finland.

Insulin and glucagon are homeostatic regulators of glucose metabolism. Insulin resistance of tissues may reduce the effect of insulin on metabolism in peripheral tissues during early lactation. Glucagon is known to stimulate gluconeogenesis and possibly lipolysis (1). Leptin concentrations in blood are positively correlated with obesity and increased energy intake and leptin is possibly a homeorhetic signal of energy status affecting feed intake (2). During three weeks before expected calving date, six multiparous cows were fed total mixed ratio according to energy requirement. After calving the cows were divided on two feeding regimes, total mixed ratio (TMR) and separate feeding (SEP) of grass silage and cereal concentrate. Average milk yields were 37.6 kg/d and 35.4 kg/d in TMR and SEP and total dry matter intakes 20.2 kg/d and 17.6 kg/d, during experimental period of six weeks. Plasma NEFA concentration was lower with TMR than with SEP and there were tendencies towards higher glucose and insulin concentrations in TMR than SEP. Milk fat content was higher in SEP than TMR. During early lactation, insulin and leptin concentrations tended to be decreased, whereas glucagon concentration and glucagon:insulin ratio tended to be higher than during late pregnancy. These data suggest that despite increased tissue resistance, insulin still has an antilipolytic role during early lactation. Glucagon had no lipolytic effect. Relatively low sensitivity (3) of assay (Linco XL-85K) may have lead to an underestimation of leptin concentrations.

		-21d	-7d	+1d	+7d	+14d	+28d	+42d
Insulin, µIU/ml	TMR	6.6	5.2	6.8	5.5	5.5	5.4	6.4
	SEP	8.3	6.1	5.3	4.3	3.5	4.5	6.7
Glucagon, pg/ml	TMR	151.9	92.9	119.6	142.1	139.8	141.7	149.5
	SEP	130.8	92.4	95.2	111.4	125.9	137.7	120.7
Glucagon/insulin	TMR	26.6	17.6	18.1	26.4	26.5	27.5	23.2
	SEP	15.9	15.7	19.1	26.7	40.3	31.4	18.3
Leptin, ng/ml HE	TMR	3.8			3.2	3.2	3.4	
	SEP	4.5			2.8	3.4	3.3	

References: Bauman and Elliot, 1983, pp. 437 – 468. In. Biochemistry of lactation (ed. T.B. Mepham); Chilliard et al., 1999, Prod. Anim. **12**, 225; Delavaud et al., 2000, J. Endocr. **165**, 519

Key Words: Cow, Insulin, Glucagon, Leptin

P44 **PLASMA THYROXINE AND 3-3'-5 TRIIODOTHYRONINE ANNUAL TRENDS IN MALE GOATS ARE AFFECTED BY SHORT PHOTOPERIODIC CYCLES.** L.Todini [1], J.A. Delgadillo[2], A. Debenedetti [3], P. Chemineau[4]. [1]*Department of Veterinary Science, University of Camerino 62024 Italy,* [2]*UAA A. Narro, 940 Torreon, Coahuila, Mexico,* [3]*Department of Biopathologic Veterinary Science, University of Perugia Italy,* [4]*INRA Département de Physiologie Animale, 37380 Nouzilly France.*

In temperate latitudes goat species shows a marked seasonal reproductive activity, with a breeding season situated during the shortest days of the year. In bucks, alternations of 1 or 2 month periods of long and short days abolished, during three consecutive years, the sasonality of endocrine and sexual activity (1). Thyroid hormones play an important (permissive) role in the expression of endogenous seasonal rhythms of neuroendocrine and reproductive activity in sheep, as in many species of birds (2). Seasonal cycles of circulating thyroid hormones have been described comparatively with testicular endocrine activity in a broad variety of other seasonal breeders (3). In order to determine

if thyroxine (T4) and 3-3'-5-triiodothyronine (T3) plasma concentrations were affected by photoperiodic changes, these hormones have been assayed in weekly plasma samples collected during one year from Alpine and Saanen bucks exposed to natural photoperiod (46°N lat, control group), or to alternations between 2 months (4M group) or 1 month (1M) of long days (16L:8D) and short days (8L:16D). In the control group the thyroid hormones showed marked seasonal variations; maximal monthly means (±sd) were reached in April for T4 (35.7±1.8 ng/ml) and in March for T3 (0.91±0.11 ng/ml), with the minimal concentrations being in October for T4 (22.8±1.0 ng/ml) and in September for T3 (0.42±0.01 ng/ml). In the 4M group T4 plasma levels varied with daylength ($p<0.05$), showing an overall mean concentration (±sd) significantly ($p<0.01$) higher during short days (37.3±7.5 ng/ml) than during long days (32.2±7.3 ng/ml), and varied with time intra-photoperiod ($p<0.0001$). There was also a significant effect of daylength ($p<0.0001$) and time ($p<0.0001$) on T3 plasma levels: in all 3 photoperiodic cycles, after the transition fron long to short days, a decrease in T3 levels was observed, and it persisted until the end of short day periods, being significant in the latter half of these periods ($p<0.05$). In the 2M group T3 but not T4 plasma concentrations were affected by both daylength ($p<0.0001$) and time ($p<0.0001$), being enhanced during long days and decreasing during short days. A delay of the effects exerted on thyroid secretory activity (T4 plasma levels) could be hypothesized, while the mechanisms of peripheral deiodation (T3 plasma levels) are more rapid. Negative correlations ($p<0.01$) were found between thyroid hormones and testosterone plasma concentration in each group of animals. It is concluded that photoperiod affects thyroid hormones plasma concentrations in male goat, since different light regimes resulted in different profiles of both T3 and T4. Results also confirmed the strong temporal links between variations in thyroid hormones and testosterone levels, even during photoperiodic cycles of short duration.

References: (1) Delgadillo *et al.*, 1993, *Reprod. Nutr Dév.* 33, 609; (2) Nicholls *et al.*, 1988, *Reprod. Nutr Dév.* 28, 375; (3) Karsch *et al.*, 1995 *J.Reprod.Fert.* suppl 49, 409.

Key words: Photoperiod, thyroxine, 3-3'-5-triiodothyronine, buck.

P45 ASSOCIATIONS BETWEEN SSCPs IN THE GH GENE AND MILK TRAITS IN "SERRA DA ESTRELA" EWES. M.R. Marques[*,**], I.C. Santos[*,**], C.C. Belo[**], A. Cravador[*]. [*] *FERN, Universidade do Algarve, Campus de Gambelas, 8000-117 Faro, Portugal, and* [**] *Departamento de Sistemas e Técnicas de Produção Animal, Estação Zootécnica Nacional, Fonte Boa, 2000-763 Vale de Santarém, Portugal.*

"Serra da Estrela" is the autoctonous Portuguese ovine breed with the best potential for milk production. Cheese is the main agricultural product of the mountainous region of "Serra da Estrela", with great economic and social importance for rural populations. The detection of genetic markers at the GH gene associated with milk traits will contribute to establish early selection criteria. Polymerase Chain Reaction - Single Strand Conformation Polymorphism (PCR-SSCP) analysis (1) of GH gene performed on 200 "Serra da Estrela" ewes revealed that this breed's GH gene is highly polymorphic: ten conformation patterns were observed in exon 2, eight in exon 3, two in exon 4 and five in exon 5; exon 1 was found to be monomorphic.

The statistical analysis of results, performed using a general linear model from SAS (2), led to the establishment of associations between some of these SSCPs found in exons 3 and 4 and quantitative variations in production traits. A positive relationship was detected between pattern B of exon 4 and milk production: ewes with pattern B (frequency of 14.8 %) produced 57 l more milked milk ($P<0.05$) and 65 l more milk per lactation ($P<0.05$) than ewes with the A pattern (frequency of 85.2 %). In relation to milk composition, exon 3 tended to be associated with milk fat content: ewes with conformation patterns E and G produced milk with higher fat content than ewes with patterns A, B or C (7.4 and 7.9 % *vs* 6.5, 6.4 and 6.2 %, respectively; $P<0.10$; frequencies are 6.0, 8.7, 52.5, 21.3 and 5.5 %, respectively for patterns E, G, A, B and C of GH exon 3).

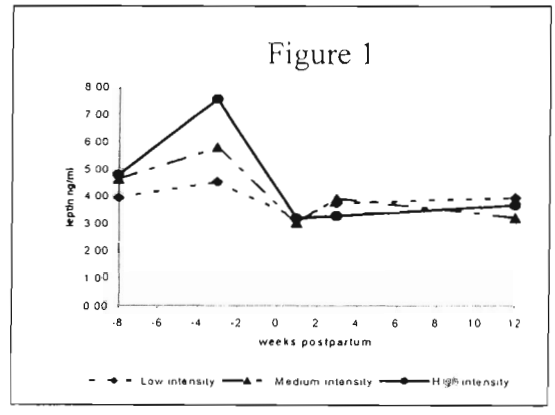
In order to confirm these results, this study was extended to a population of 500 ewes. Blood was collected from ewes owned by "Associação Nacional de Criadores de Ovinos Serra da Estrela" (ANCOSE) associates. Genomic DNA was extracted from blood samples. Fragments of 112, 198, 154, 200 and 289 bp containing GH exons 1, 2, 3, 4 and 5 respectively, were amplified by PCR. After chemical and thermal denaturation, single-stranded PCR products were loaded onto non-denaturing polyacrylamide gels and run under optimised conditions. Gels were silver stained (3). Statistical analysis will be performed as described above. (We acknowledge "Fundação para a Ciência e a Tecnologia" (FCT) (PRAXIS XXI 3/3.2/CA/1991/95) for financial support and ANCOSE for providing zootechnical data. M.R.Marques and I.C.Santos thank FCT for PhD grants SFRH/BD/1140/2000 and BD/18061/98.)

References: (1) Orita *et al.*, 1989, *Genomics*, 5, 874-879; (2) SAS, 1989, SAS® User's Guide: Statistics, Version 6 Ed. SAS Inst., Inc. Cary, NC, USA; (3) Oto *et al.*, 1993, *Analyt. Biochem.* 213, 19-22

Key Words: oGH, PCR-SSCP, genetic markers, milk traits.

P46 **EFFECT OF DRY PERIOD FEED INTAKE ON PLASMA LEPTIN IN DAIRY COWS.** K. Holtenius, S. Agenäs,* C. Delavaud, Y. Chilliard** *Swedish University of Agricultural, Sciences S 750 07 Uppsala, Sweden **Unite de Recherche sur les Herbivores, INRA, Theix, 63122 St-Genes-Champanelle, France.

Leptin, a protein mainly secreted from the white adipocytes has been implicated in the regulation of food intake, energy expenditure and whole body energy balance. The expression and secretion of leptin is highly correlated with body fat mass and adipocytes. The regulation of leptin expression and release is probably mediated in part by insulin, at least in rodents and humans. The aim of the present study was to provide information of how different levels of feed intake during the dry period affected the plasma level of leptin and insulin during the dry period and in early lactation. Multiparous dairy cows (n=24) were randomly allocated in three experimental groups. All groups were fed a total mixed ration (TMR) of the same composition during the dry period (8 weeks). The amount of MJ ME/day varied between the experimental groups. (70MJ =low;105MJ = medium; 170=High). After parturition all cows were fed a TMR ad lib. for 12 weeks. Feed intake, milk yield, body weight and body condition scoring (BCS) were registered. Leptin was measured with a RIA-method evaluated for bovine leptin (Delavaud et al., 2000). Leptin in plasma was significantly higher in the cows fed the high diet as compared to the other diets 3 weeks prior to parturition. Cows receiving the low diet had the lowest leptin level (Fig 1). Immediately after parturition the leptin level decreased. The dry period feed intake did not affect plasma leptin when the cows were lactating. However the feed intake in early lactation was significantly reduced in the cows which received the high intensity diet during the dry period and they went into a deeper negative energy balance. Leptin was correlated to insulin during the late dry period ($p<0.001$; $R^2=0.50$). Furthermore leptin correlated to the change in BCS during the dry period ($p<0.004$; $R^2=0.37$). In conclusion, plasma leptin in dairy cows was related to nutrition, BCS and insulin in the dry period. After parturition the voluntary feed intake was reduced in cows fed a high intensity diet during the dry period but leptin did not differ between treatments.

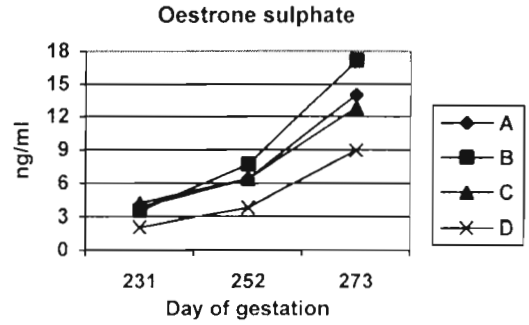


References: Delavaud et al., 2000 J. Endocrinol. **165**, 519-26.

Keywords: Bovine Leptin Dry period Nutrition

P47 **PRELIMINARY OBSERVATIONS ON OESTRONE SULPHATE IN LATE PREGNANCY OF PIEDMONTESE CATTLE AND RELATIONSHIPS WITH CALF BIRTH WEIGHT.** M.G. Terzano, F. Abeni*, G. Bergoglio*, G. Masoero*, A. Borghese. *Breeding Technology Section and *Operative Section of Turin, National Institute of Animal Production, I-00016 Monterotondo Scalo, Rome, Italy.*

Oestrone sulphate (E_1S) is considered a reliable signal of foetal-placental unit activity (1). Piedmontese is a beef breed with a high frequency of muscular hypertrophy gene and calving difficulty is a problem considered in management and selection. However, also an excessively light calf is undesirable, because frequently of poor viability. In a study supported by Piedmont Region, E_1S was determined in blood plasma of 59 gestating Piedmontese cows (15 primiparous and 44 pluriparous). Samples were taken three times, every 21 d, before feeding, starting at the end of the 7th mo of gestation. Raw data were not normally distributed, so they were processed after logarithmic transformation. Preliminarily, effect of gestation number (primiparous or multiparous) was tested, evidencing no effects on E_1S level and pattern. In order to evaluate if a different foetal weight could be preliminarily classified, the dams were divided into four classes, with a newborn calf weight of 51 or more (A), from 46 to 50 (B), from 41 to 45 (C) and 40 or less (D) kg of body weight. Newborn calf weight class and days of gestation had a significant effect on E_1S without interactions. There was also a significant effect of the dam, confirming the high variability of E_1S among animals of a same group. E_1S significantly increased for each group from 231 to 273 d of gestation (see Fig); the reported values are in agreement with those of literature (1, 2). The logarithm of plasma E_1S in group D was significantly lower than those of the other groups starting at 231 d of gestation ($P < 0.05$; see Fig). There were not differences in E_1S level between dams with an easy or difficult calving. From these data, it is not clear if it could be possible to predict newborn calf of a weight higher than 50 kg. However, it seems more reliable to adopt plasma E_1S level as a tool to evidence problems during gestation that can lead to a newborn lighter than 40 kg, that is a weight that could be associated to poor viability in the first days of life. In addition, the availability of a reliable test at the end of the seventh month of gestation allows a nutritional regulation in the last weeks before calving. However, further studies with more animals will be necessary to confirm these preliminary results.



References: Zhang *et al.*, 1999, *Anim. Reprod. Sci.* **54**, 169. Echternkamp, 1993, *Anim. Reprod. Sci.* **32**, 1.
Key Words: Oestrone Sulphate, Beef Cattle, Piedmontese Breed, Calf Weight

P48 **MAIN ENDOCRINE-METABOLIC DIFFERENCES BETWEEN 1st AND 2nd LACTATION OF THE SAME DAIRY COWS. FIRST RESULTS.** *G. Bertoni*, E. Trevisi, R. Lombardelli, F. Piccioli-Cappelli. *Istituto di Zootecnica, Faculty of Agriculture, U.C.S.C., 29100 Piacenza, Italy (bertoni@pc.unicatt.it).*

The lower milk yield in primiparous dairy cows compared with following lactations is mainly attributed to a lower development of the mammary gland. Nevertheless, the role played by mammary gland (is it pushed or does it pull?) and by endocrine system is not totally clear jet. With the aim to study the metabolic-endocrine changes that justify the marked differences in terms of milk yield between 1st (L1) and 2nd (L2) lactation, 2 heifers Italian Friesian were followed from the last month of 1st pregnancy until the end of 2nd lactation. The animals were housed and fed in very similar conditions in the artificially ventilated and lighted barn of the Institute. They were individually fed with 2 similar forage meals (corn silage, chopped alfalfa and grass hays), every 12 h, and 2 (dry period) or 8 (lactating period) meals of appropriate concentrate, distributed at regular intervals. The cows were continuously checked for: health status, daily dry matter intake (DMI) and milk yield at every milking. Blood samples were withdrawn before the morning meal every 3-4 days during the whole trial period. In addition, blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 9, 11, 24 hours from morning forage meal, weekly from –28 to 28 days in milk (DIM) and fortnightly till 120 DIM to determine glucose, urea, non-esterified fatty acids (NEFA), ß-hydroxybutyrate (ß-OHB), creatinine, GH, insulin, T3. Two-ways ANOVA (time, calving and their interaction) was used for statistical evaluation. DMI in the 2 lactations was markedly different in all the stages (about 6-7 kg/d higher in L2), although the behaviour of the ingestion was analogous. The milk yield showed a quicker increase in L2 cows and the peak was reached earlier (35 vs 55 DIM). GH peaked between 7 and 14 DIM in both lactations and afterwards decreased, was higher in L1 (5.2 vs 4.6 ng/ml). Insulin showed an opposite behaviour of GH, with marked reduction after calving (nadir at 7 DIM, about 6.5 µU/ml), followed by a progressive increase during lactation. Levels were similar between parities and only after 60 DIM lower in L1. Level and behaviour of T3 were similar between parities, but after 30 DIM the increase was higher in L1. After calving, glucose decreased while ßOHB and NEFA increased, in both lactations, but level of glucose was constantly higher in L1 as well as ß OHB and NEFA. Moreover, L1 showed a higher level of creatinine in dry period, but similar after 14 DIM. Level of urea was similar in dry period and always higher in L2 after calving. Cows in L1 seems to have a more favourable endo-crine condition (i.e. higher GH) for milk synthesis; also higher is the availability of nutrients (i.e. glucose and NEFA), nevertheless they do not seem fully utilized by the mammary gland. Conversely, cows in L2 showed lower nutrient blood concentrations, despite lower GH and higher DMI. This suggests that other factors affect mammary gland nutrient uptake (cells number, IGF, paracrine factors, etc.) and in turn it determines the different blood level of nutrients. From these preliminary results it seems that any of metabolic-endocrine differences observed can fully justify different milk yield between L1 and L2, indicating that mammary gland activity could be of major importance.

Key Words: milk yield, parity, hormones, dairy cows.

P49 ENDOTOXIN EFFECTS ON METABOLIC-ENDOCRINE STATUS OF DAIRY COWS PREVIOUSLY TREATED WITH rbST. E. Trevisi, F. Piccioli-Cappelli, R. Lombardelli, G. Bertoni. *Istituto di Zootecnica, Faculty of Agriculture, U.C.S.C., 29100 Piacenza, Italy*

Endotoxin treatment – used as a model of inflammatory event - has determined the delay of milk yield increase when injected contemporary with recombinant bovine somatotropin (rbST). The mechanisms involved could be fever, anorexia and metabolic disorders due to the cytokines, which stimulate the liver to synthesise the acute phase proteins instead of the usual ones, IGF included. Aim of the present work was to check the endotoxin effects when rbST treatment had already determined a substantial milk yield increase. Four dairy cows in average-late lactation were used for each treatment: rbST (Ely Lilly, 25 mg/d), rbST+endotoxin (0.1 μg/kg b.w. from E. coli, sero-type 055:B5), pyrogen free water (control). Each treatment was performed 1 hour after the morning forage meal. Control and rbST groups were treated for 8 days, whereas rbST+endotoxin cows received rbST for 10 consecutive days and endotoxin at 7th and 8th day of that challenge. Blood samples were collected at 0, 1, 2,5, 4, 7, 10, 13, 24 hours from the morning forage meal, on day before, day 1,2,3,7,8 and 20 from the start of treatments to determine metabolic profile as well as GH, IGF-I, insulin, T3, rT3, cortisol, glucagon. Milk yield and dry matter intake (DMI) were monitored daily. Two-ways ANOVA (time, treatment and their interaction) was used for statistical evaluation. rbST progressively enhanced the milk yield, the peak was of +15%. The endotoxin injections during rbST treatments caused a significant drop of milk yield, under the pre-rbST treatment values. The recovery of milk yield began already during the 2nd endotoxin treatment and was completed in the 2 next days. Nevertheless, the reduction observed in the 10 days following the suspension of rbST injections was more marked in rbST+endotoxin group (–18 vs -12%). DMI was slightly reduced (-6%) only during the 1st endotoxin treatment day. Daily rbST treatments, besides the raise of GH ($P<0.05$ until 12 hours after the injection), promoted a significant increase of glucose, NEFA, insulin, IGF-I and a significant decrease of urea and glucagon. Most of these changes were gradual during the 1st part of treatment period. Endotoxin injection caused a marked and short-lived increase of cortisol, glucagon, rT3 as well as the reduction of T3, and a prolonged increase of insulin. After 2nd endotoxin treatment, the magnitude of all the blood variations was less pronounced, except for insulin and glucose; the latter was slightly reduced after 1st injection but increased after the 2nd one ($P<0.05$). Moreover, IGF-I showed only a short-lived reduction after both endotoxin treatments. Therefore, cows treated with rbST+endotoxin reduced milk yield although GH, IGF-I and nutrients availability (i.e. NEFA and glucose) showed high levels, likely due to the strong raise of cortisol, insulin and glucagon and to the reduction of T3 and, perhaps, DMI. On 2nd day of treatment, when except insulin the blood values returned to previous levels, the milk yield started the recovery. For these reasons the decrease of milk synthesis seems mainly due to a different nutrients partition, caused by different endocrine pattern. These temporary impairment of endocrine balance cannot justify the higher reduction of milk yield after the end of rbST treatments, that could be due to a cytokine damage of cells, followed by a gradual loss of yield when nutrients availability is lower.

Key Words: endotoxin, rbST, hormones, dairy cows.

P50 A NEW CONCEPT FOR VASCULAR CONTROL OF LUTEAL FUNCTION IN LUTEOLYSIS IN THE COW: THE IMPACT OF ENDOTHELIN-1, ANGIOTENSIN II AND PGF2α. A. Miyamoto, K. Hayashi, T.J. Acosta, S. Kobayashi, D. Schams*, M. Ohtani. *Obihiro Univ of Agric & Vet Med, Obihiro, 080-8555 Obihiro, Japan, and *Institute of Physiology, TU-Munich, D-80350 Freising, Germany.*

Prostaglandin F2 (PGF2α) is the primary luteolysin in the cow. During PGF2α-induced luteolysis, the blood flow decreases in the ovarian artery, and the endothelin-1 (ET-1) secretion within the corpus luteum (CL) increases, suggesting a close relationship between the luteal blood flow and ET-1 in luteolysis. Recently, we found that angiotensin II (Ang II) may also regulate luteolysis. Thus, this study was aimed to evaluate the impact of ET-1, Ang II and PGF2α in luteolysis by using 3 different *in vivo* models as follows: 1) a microdialysis system (MDS) surgically implanted in the midcycle CL to determine the local release of peptides and PGs, 2) a color and pulsed Doppler ultrasonography to determine the blood flow within the CL, and 3) a model based on an intraluteal injection of ET-1 or Ang II at 30 min after a subluteolytic PG injection i.m. 1) In the MDS study, a luteolytic PG injection induced a rapid increase in the release of ET-1 as well as Ang II, PGF2 and PGE2 within the regressing CL after 4 h when plasma progesterone (P) concentration had already decreased. The ET-1 release kept high levels up to 72 h after PG i.m., while the Ang II release showed high levels only during the first 8 h. The release of both PGs dropped to the basal levels 8 h after PG i.m., but increased again between 48-72 h. 2) In the study using a color Doppler ultrasonography, the blood flow within the midcycle CL after PG injection firstly increased at 0.5-2 h, decreased at 4 h to the same levels observed at 0 h, and then further decreased to lower levels from 8-48 h. The time averaged maximum velocity of the blood flow and the CL volume decreased at 8 h and further decreased to 24 h, indicating a structural luteolysis. These changes were

not detected in the early CL (Day 4) in which luteolysis did not occur. 3) In the final study, an intraluteal injection of ET-1 or Ang II after subluteolytic PG (1/4 dose) i.m. resulted in the rapid reduction of plasma P concentration similar as the PG full-dose (ET-1: 1-2 ng/ml, Ang II: 0.5 ng/ml; PG full-dose: 0.5 ng/ml). The ET-1 treated cows did not show estrus during 5 day after treatment, whereas the Ang II-treated cows showed estrus after 5 days, suggesting different actions on the CL function. In contrast, neither a PG full-dose nor ET-1 after PG i.m. on Day 5 had clear effect on CL development. Overall results support the concept that the interaction of ET-1 and Ang II with PGF2α induces the functional luteolysis in the cow. A novel acute increase of blood flow in the CL occurred at 0.5-2 h after PG i.m. may trigger the cascade of luteolysis. The lack of intraluteal vascular response to PG in the early CL appears to directly correlate to the PG-resistsant ability. *Supported by JSPS grants.*
Key Words: Luteolysis, Blood flow, Endothelin-1, Angiotensin II

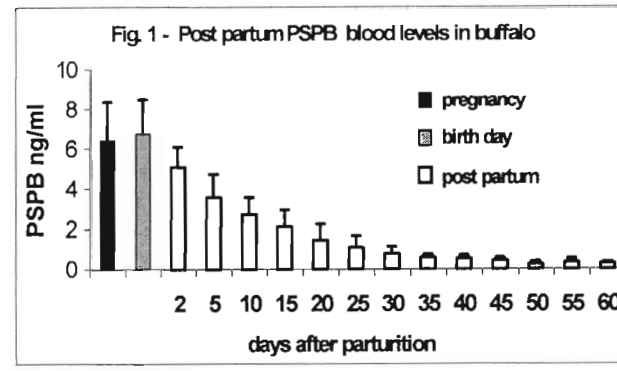
P51 **EFFECTS OF AN ESTROGEN TREATMENT ON THE TISSUE SPECIFIC EXPRESSION OF STEROID RECEPTORS IN THE BOVINE GASTROINTESTINAL TRACT: QUANTIFICATION OF ANDROGEN RECEPTOR (AR), PROGESTERONE RECEPTOR (PR), ESTROGEN RECEPTORS (ER) AND MRNA WITH REAL-TIME RT-PCR.** Pfaffl MW, I Lange, A Daxenberger & HHD Meyer; *Institute of Physiology, Center of Life and Food Sciences, Technical University of Munich, D-85350 Freising-Weihenstephan, Germany*

We have examined the tissue specific mRNA expression of AR, PR, ER and ER in the bovine intestinal tract using real-time RT-PCR. Goal of this study was to evaluate the deviating tissue sensitivities and the influence of the estrogen active preparation RALGRO on the regulation of steroid receptors in gastrointestinal tract. RALGRO contains Zeranol, a derivative of the mycotoxin Zearalenon, which shows strong estrogenic and anabolic effects. It exhibits all symptoms of hyper-estrogenism in particular reproductive and developmental disorders. Eight heifers were treated over 8 weeks with multiple pellet implantations (0x, 1x, 3x, 10x) and after slaughtering Zeranol concentration were measured by enzyme-immuno-assay. Plasma and tissues Zeranol concentrations were elevated equivalent to the multiple treatment. To quantify low abundant transcripts sensitive and reliable real-time RT-PCR quantification methods were developed and validated on the LightCycler. Four stomachs (rumen, reticulum, omasum, abomasum) and six different gut regions (duodenum, jejunum, ileum, caecum, colon, rectum) were examined. AR, PR, ER and ER mRNAs were detected in all tissues. The AR mRNA was the major transcript in the 3 fore stomach (rumen, reticulum and omasum), whereas both ER subtypes were higher abundant in abomasum and all gut compartments. PR mRNA was very low abundant in all investigated tissues. In all stomachs and duodenum the expression ratio (R) of ER/ER was high ($29>R>6.5$) and low ($R<1$) in ileum and rectum, where ER was higher concentrated. With increasing Zeranol concentrations a significant up-regulation of ER mRNA expression could be observed in abomasum ($r=0.72$; $p<0.05$) and a down-regulation in jejunum ($r=0.77$; $p<0.05$). For ER mRNA an up-regulation was shown in rectum ($r=0.96$; $p<0.001$), a down-regulation in jejunum ($r=0.72$; $p<0.05$) and a trend of down-regulation in reticulum ($r=0.68$; $p=0.06$). PR mRNA was up-regulated in omasum ($r=0.92$; $p<0.001$) and down-regulated in jejunum ($r=0.73$; $p<0.05$). For AR mRNA expression no significant correlation with increasing Zeranol concentrations could be observed. In conclusion, our expression results indicate the existence of AR, PR and both ER subtypes in bovine gastrointestinal tract. Gastrointestinal tissues compartments exhibit a tissue specific expression pattern for steroid receptors and a tissue specific regulation under estrogen treatment. Therefore the stomachs and the gut are possible target for steroid hormone action. Beside this these data support the hypothesis, that ER may have different biological functions than ER.
Key words : Zeranol, estrogen treatment, AE; ER, ER, real-time RT-PCR.

P52 **PSPB (PREGNANCY SPECIFIC PROTEIN B) PLASMA LEVELS DURING POST-PARTUM IN BUFFALO COWS (*Bubalus bubalis*).** Malfatti A., Debenedetti A.[1], Barbato O.[1],Mancini L.[1], Terzano M.G.[2], Barile V.[2], Humblot P[3]. *Department of Veterinary Science. Faculty of Veterinary Medicine, Camerino (Italy)* [1] *Department of Biopathologic Veterinary Science. Faculty of Veterinary Medicine, Perugia (Italy)* [2] *Istituto Sperimentale per la Zootecnia, Roma (Italy).* [3] *UNCEIA, 13 rue Jouet, 94703 Maisons Alfort (France).*

Pregnancy specific protein B (PSPB), synthesized by the binucleate placental cells, is detectable in circulating blood of pregnant cows by means of a specific RIA, which is now widely used as pregnancy test. An analogous pregnancy protein has been demonstrated by the same method in buffalo cows (1). In both species PSPB becomes well detectable in maternal blood 30 days after fertilization. In the bovine PSPB is increasing during pregnancy, reaching very high levels at birth and, due to the very slow post-partum disappearance of the protein from circulating blood, a PSPB pregnancy diagnosis by RIA in cows must be delayed at least 100 days after birth (2). On the contrary, in buffaloes the

PSPB concentrations are lower and stay relatively constant till birth (1). Aim of the research was to clarify the post-partum trend of PSPB blood level in buffalo cows, in order to define the temporal limits of a reliable PSPB pregnancy test in this species. Blood samples were taken from 13 pregnant females at the end of pregnancy, at birth and then at 5 day intervals for 60 days. The plasmas obtained were assayed at the UNCEIA laboratory of Maisons Alfort (France) by means of a double antibody RIA, as described by (3). The results are reported in Fig. 1. The PSPB was present in all pregnant animals. Blood levels were not significantly different at the end of pregnancy and at birth date. The post partum persistence of the buffalo PSPB in circulating blood was confirmed to be very long, the concentrations halving 8-10 days post-partum (that is of the same order as reported for cows). However, from the 50th day after birth the residual PSPB blood levels are so low (<0.3ng/ml) that from this day, pregnancy diagnosis based on PSPB assay seems perfectly reliable.



References: (1) Debenedetti et al., 1997 *Proc. V World Buffalo Congr.*, 771; (2) Humblot et al., 1988 *J.Reprod.Fert.*, **83**, 215; (3) Humblot, 1992 *Ann. Zootech.*, **41**, 389.

Key words: Buffalo, Pregnancy diagnosis, PSPB.

P53 PREGNANCY-ASSOCIATED GLYCOPROTEIN DETECTION BY RIA IN PREGNANT BUFFALO COWS (*Bubalus bubalis*). COMPARISON OF THREE DIFFERENT ANTISERA.

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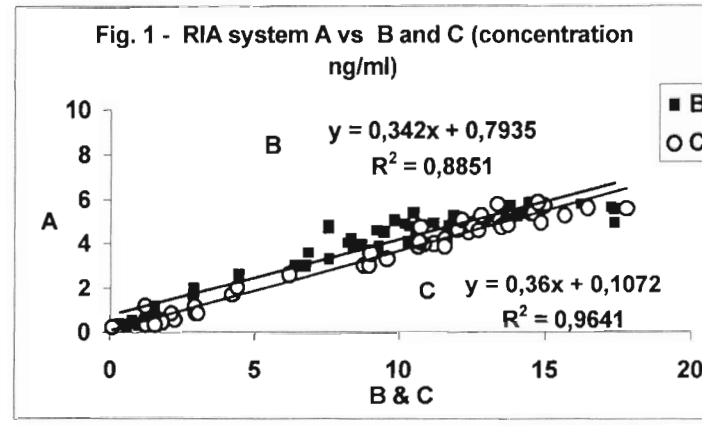
The Pregnancy Associated Glycoproteins (PAGs), synthesized by trophoblastic cells, have been isolated from several ruminant species. They are released in maternal blood circulation where they are detectable by RIA from a few weeks after fertilization till parturition and even after. In cattle, the measurement of PAG (or PSPB) concentrations is now widely used as pregnancy test. In a preliminary investigation, a PSPB RIA system was applied in buffalo cows but the measured concentrations were much lower than in the bovine species, probably due to a lower affinity towards the antibodies. The aim of this work was to compare the efficiency of three different PAG antisera in order to improve the accuracy of assays in buffalo cows. Plasma samples (n=52) collected from 22 buffalo cows at different periods of pregnancy and postpartum have been tested by three RIA methods using antisera raised against different PAG preparations: System A = bovine PAG_{67} (boPAG-1), System B = caprine PAG_{55+59} and System C = caprine PAG_{55+62}. In the three systems the boPAG-1 was used as standard (0.2-25 ng/ml) and tracer. The regressions and the variance analysis were calculated by using the SAS.



All the three RIA systems demonstrated to be able to measure the buffalo PAG concentrations (> 1,0 ng/ml as discriminant) for pregnancy diagnosis. From the regressions in fig.1, the values of RIA A were significantly ($P<0.01$) lower than those of systems B and C, the last one giving the highest concentrations (near three times higher than A). The correlation coefficient (r^2) between A and C was higher (0.964) than between A and B (0.885) and between B and C (0.915) systems. These results suggest that the buffalo pregnancy proteins are better recognised by the antisera raised against the caprine PAGs.

Key words: **Buffalo, Pregnancy, PAG.**

P54 **ORGANOCHLORINES AFFECT PLASMA CORTISOL IN POLAR BEARS (*URSUS MARITIMUS*).** I C Oskam[1], A E Derocher[2], E Lie[1,3], J Utne Skaare[1,3], Ø Wiig[4], S Larsen[1], E Dahl[1], E Ropstad[1] [1)] *The Norwegian School of Veterinary Science, N-0033 Oslo, Norway,* [2)] *Norwegian Polar Institute, N-9296 Tromsø, Norway,* [3)] *National Veterinary Institute, N-0033 Oslo, Norway,* [4)] *Zoological Museum, University of Oslo, N-0562 Oslo, Norway*

The polar bear is among the most highly organochlorine-contaminated species of the arctic mammals. Exceptionally high levels of the lipophilic and persistent polychlorinated biphenyls (PCBs) and a group of pesticides accumulate in adipose tissue of this species. Cortisol is virtually involved in every organ and tissue in the body. There is growing concern that organochlorines (OCs) are able to alter basic endocrine pathways and thereby influence the functions of many biological systems. From 121 male- and 130 female polar bears, which were captured and anaesthetized prior to sampling, samples were collected in March, April, May and August in the period from 1995 until 1998 in the Svalbard region. General Linear Models was used to assess the relationship between plasma cortisol and OC pollution level. Dependent variable: plasma cortisol; independent variables: age, girth, plasma fat and single OCs. The data were corrected for the effect of season and each gender was analysed separately. The concentrations of 6 pesticides, 16 PCB congeners and cortisol were determined in blood plasma. The male model explained 42.7 % and the female model 30.5 % of the total cortisol variation. In males, oxychlordan and PCB-99 were associated with a decrease in plasma cortisol while PCB-187 and PCB-153 were associated with increasing cortisol. In females, PCB-99 was associated with a decrease in plasma cortisol while PCB-187 and PCB-153 were associated with increasing cortisol concentration. In males, the PCB congeners 99, 153, 187 and 194 explained 29.6 % of the total variation in cortisol explained by the model, while oxychlordan explained 6.5 %. In females, the PCB congeners 99, 153 and 187 explained 38.2 % of the total variation in cortisol explained by the model.
Key words: Polar bears, cortisol, organochlorines.

P55 **LH AND ESTROGEN (A AND B) RECEPTOR EXPRESSION IN THE MALE FOWL.** H. Tang, R. Okimoto, C Hsu and J.D. Kirby, *Dept. of Poultry Science, University of Arkansas, Fayetteville, AR 72701 USA*

The ontogeny of expression of the LH receptor gene was studied in chicken testis from hatch to sexual maturity. A cDNA probe encoding the extracellular domain of the LH receptor was used to detect the expression of LH receptor mRNA in testis. The Northern blots showed the presence of three transcripts, a major 2.7 kb band and minor 7 kb and 1.5 kb bands. The expression level of major transcript started to increase after hatching and peaked by week 2, then decreased, reaching it's nadir by week 7. The low level of expression was maintained until week 16 before increasing again. The change of LH receptor expression may reflect the development and differentiation of Leydig cells, which are required for the secretion of testosterone. ERa and ERb cDNA probes were made from PCR fragments on the region E (ligand binding domain) of chicken ERa and ERb genes respectively. To detect their sites of expression, total RNAs from chicken hypothalamus, ovary, pituitary and testis were extracted and hybridized to the ERa and ERb probes. Northern blots demonstrated that both ERa and ERb were expressed in all four tissues, but ERa was predominantly expressed in pituitary while the ERb expression level was the highest in testis but the variation was not great between tissues. Only a 7.8-kb transcript was detected in ERa, however, two transcripts with sizes of 7.8 kb and 4.9 kb were found in ERb, and both bands were observed in hypothalamus, pituitary and testis but not the ovary, in which only the 4.9 kb species appeared. Multiple transcripts and expression pattern varying with tissues in ERb suggest that the regulation mechanism of ERb is different from ERa. Further, an RT-PCR experiment confirmed the expression of ERa and ERb in hypothalamus, ovary, pituitary and testis but did not detect any ERa and ERb expression in purified chicken spermatocyte and spermatid cell populations, suggesting that ER expression is limited to testis somatic cells.
Key words: fowl, LH, estrogen, receptor

P56 **EFFECT OF SELECTION FOR MILK YIELD ON THE SOMATOTROPIC AXIS OF THE HOLSTEIN COW: ENDOCRINE PROFILES AND HEPATIC GENE EXPRESSION.** B. A. Crooker, W. J. Weber, L. S. Ma. *Department of Animal Science, University of Minnesota, St. Paul. USA.*

Cows from a breeding project initiated in 1964 to develop a stable control line (CL) that represents US breed average in 1964 and a select line (SL) that represents contemporary US Holsteins were used to evaluate changes in endocrine profiles and hepatic mRNA. Milk yield of the lines currently differs by more than 4,500 kg/305 d lactation. Lines were housed together in a free stall barn and fed ad libitum. Blood and hepatic biopsy samples were obtained from multiparous (MULT, 10 CL and 13 SL) and primiparous (PRIM, 8 CL and 8 SL) cows at -12 1, 20 0.4, and 68 0.5 d

postpartum (PP). Serum IGF-I was determined by RIA. Expression of the GH receptor (GHR), IGF-I, IGF-BP3 and IGF-BP5 genes were determined (ribonuclease protection assay). Expression of GAPDH, β-actin, and cyclophilin were evaluated as controls to correct for variation in mRNA precipitation and loading. The mRNA results are presented as pixel density relative to controls. Data were analyzed as repeated measures using PROC MIXED of SAS. Results differed when $P < 0.05$. Milk yield of PRIM and MULT CL was 18.7 and 28.4 kg/d while PRIM and MULT SL produced 29.9 and 44.6 kg/d. Serum IGF-I was greater in CL than SL (139, 118 ng/ml) and there was a parity by day interaction with a greater PP reduction in MULT (152, 89, 109 ng/ml) than PRIM (163, 121, 133 ng/ml). During this period, PP expression of hepatic β-actin was constant while GAPDH decreased and cyclophilin increased. Thus, mRNA data are reported relative to β-actin. Expression of IGF-I (0.30^{a}, 0.16^{b}, 0.25^{a}), IGF-BP3 (0.33^{a}, 0.27^{b}, 0.29^{ab}), and the liver specific GHR-1A (0.71^{a}, 0.46^{b}, 0.62^{a}) were less at 20 d PP than at -12 d PP and returned to prepartum amounts by 68 d PP. Expression of IGF-BP5 (0.14^{b}, 0.23^{a}, 0.18^{c}) increased PP while the nearly ubiquitous GHR-1B mRNA was not altered by day PP. Parity did not affect expression of these genes. The SL and CL cows expressed similar amounts of GHR-1A, IGF-BP3 and IGF-BP5 but SL cows tended to express more ($P=0.07$) IGF-I (0.27, 0.21) than CL cows. There was a line by parity interaction for GHR-1A mRNA with less in MULT SL than CL (0.48, 0.69) but similar expression in PRIM SL and CL (0.62) cows. Selection for milk yield has reduced expression of GHR-1A in MULT cows, has had no affect on hepatic IGF-BP3 or IGF-BP5 mRNA, and has tended to increase hepatic IGF-I mRNA during the periparturient period. In contrast, selection has decreased serum IGF-I.

Key Words: Genetic selection, Liver, mRNA, Somatotropic axis

P57 IDENTIFICATION OF POLYMORPHISMS RELATED TO QUANTITATIVE TRAITS IN THE PROMOTER REGION OF THE BOVINE GROWTH HORMONE GENE J. Jr. Suzuki, M.I.T. Ferro*, L. R. Furlan**, A. C. Silveira**,H.N. Oliveira**. **Faculdade de Ciências Agrárias e Veterinárias, Unesp, 14870-000, Jaboticabal, Brazil and **Faculdade de Medicina Veterinária e Zootecnia, Unesp, 18610-000, Botucatu, Brazil.*

Polymorphisms in the promoter region of the bovine growth hormone gene (bGH) were studied in 147 females of a 3/4 Aberdeen Angus/Nelore mixed breed cattle to estimate the possible association of alleles with birthweight, weaning weight serum concentration of IGF-I and other traits. For this study body weight data were collected at birth and weaning. Additionally, correlations of weight at birth and weaning and the estimated breeding values of the parental bulls were calculated. IGF-I serum concentrations were measured by RIA. DNA was extracted from the leucocyte "buffy coat" blood samples, and the polymorphisms were detected by Single Strand Conformation Polymorphism (SSCP) analysis. Two DNA fragments (GH1 and GH2) were generated by polymerase chain reaction (PCR) method, covering the whole GH gene promoter region. The GH1 fragments were represented by single basis polymorphisms and named GH1.1, GH1.2 and GH1.3, respectively. The GH2 fragments were represented by two polymorphisms named GH2.1 and GH2.2, respectively. Statistical analysis proved that GH2.2 fragment was significantly related ($P<0,05$) to IGF-I serum concentrations. The results suggest that the GH2.2 polymorphism may be useful as a marker for IGF-I serum levels.

References: Gordon et al. Molecular and Cellular Endocrinology, 33: 81, 1983. Yao et al. Genetics, 144: 1809, 1996.

Key Words: bovine growth hormone, IGF-I serum levels, gene sequence, polymorphism.

P58 INSULIN EFFECTS ON EXPRESSION OF GLUCOSE TRANSPORTERS AND GLYCOLYTIC KEY ENZYMES IN IN VITRO DERIVED BOVINE BLASTOCYSTS. *Robert Augustin* [1], Paola Pocar [1], Christine Wrenzycki [2], Heiner Niemann [2] & Bernd Fischer [1]. *[1]Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Halle (Saale), Germany, and [2] Department of Biotechnology, Institut fur Tierzucht und Tierverhalten (FAL), Mariensee, Germany*

Glucose is essential for mammalian blastocyst formation and development. It is taken up by the embryo by specific glucose transporters (Glut). Ten transporter isoforms are currently known. They share common structure features but show different kinetics in glucose transport and different tissue localisations. Insulin regulates expression of glucose transporters and membrane localisation of two isoforms, Glut4 and Glut8. Glucose transporter isoforms were found to be developmentally expressed during in vitro bovine preimplantation development. Insulin increased glucose uptake and had effects on blastocyst development. Employing semiquantitative RT-PCR we have investigated short and long term effects of insulin on mRNA expression of glucose transporter isoforms 1, 3, 8 and the glycolytic enzymes hexokinase and phosphofructokinase in in vitro derived bovine zygotes and blastocysts. Eight days old blastocysts were treated for 1, 2 or 4 hours with 10µg/mL insulin. Zygotes cultured to the blastocyst stage in insulin supplemented

medium (10µg/mL) were analyzed eight days after insemination. One hour exposure of blastocysts to insulin resulted in increased mRNA expression for Glut3 and Glut8 and the two enzymes investigated ($p<0,05$), while Glut1 transcription did not differ significantly. Two and four hours exposure had no significant effects. Insulin supplementation during in vitro culture did not influence the transcription of any of the genes studied at the blastocyst stage. However, elevated blastocyst cell numbers and a higher rate of hatched blastocysts were observed. The ratio of trophectoderm to embryoblast cells did n9ot differ significantly between the two groups. We conclude that bovine preimplantation embryos are insulin-sensitive in terms of metabolic and mitogenic effects. Further investigations will be necessary to prove whether bovine embryos cultured with insulin are of higher quality and which insulin signaling pathways are involved in insulin action.

Key Words: glucose transporter, glycolysis, insulin, bovine, embryo culture, in vitro.

P59 ***IN VITRO*** **EFFECT OF PROLACTIN ON 5'-DEIODINASE IN BOVINE MAMMARY GLAND.** C. Pezzi, P.A. Accorsi, R. Gaiani. *University of Bologna, 40064 Bologna, Italy.*

Thyroid hormones are important for maintaining normal lactation. The metabolically active thyroid hormone (T3) is produced by enzymatic 5'-deiodination of T4 in both thyroid and extrathyroidal tissues. The extrathyroidal activity of thyroxine 5'-deiodinase (5'D) is an important control point for regulating the thyroid status of animal tissues in various physiological situations. In rats 5'D activity increases in mammary gland proportionally to lactational intensity to maintain the mammary gland in an euthyroid status (2). In mammals PRL regulates the development of the mammary gland and has evolved the specialised function of stimulating milk synthesis and secretion, but its role varies from one species to another. In vivo studies have demonstrated that PRL elicits a discrete increase in mammary 5'D activity in rats without modifying the mRNA content (1). In contrast there are inadequate knowledge about the relationship between prolactin and 5'D activity in lactating bovine mammary gland. Mammary explants, obtained from Italian-Friesian non-pregnant cows in mid lactation, were incubated at 37°C (95% air; 5% CO_2) with Medium 199 supplemented as follows: treatment 1) Medium 199 alone (control); treatment 2) Medium 199 supplemented with different concentration of PRL (50, 250, 500, 1000 ng/ml). After 30 minutes and 24 hours of culture we evaluated activity of thyroxin 5'-deiodinase, using a modification of the method of Leonard and Rosenberg (1980) (3) and expression of 5'D mRNA by reverse transcription-polymerase chain reaction (RT-PCR). Oligonucleotide primers used in the RT-PCR assay were derived from the sequence of Rattus norvegicus type II iodothyronine deiodinase mRNA, complete cds (accession U53505, GenBank DNA sequences), chosen in the conserved region aligning rat and human. 5'D activity was expressed as pmol/mg protein/h and 5'D mRNA was expressed in arbitrary units (optical density - O.D./mm^2) and normalised using the signals generated with ß-actin. Statistical analysis of the results was performed using the one way ANOVA method.5'-deiodinase expression and activity observed in mammary explants cultured with only Medium 199 were 147.33 O.D./mm^2 and 9023.7 nmol/mg protein/h at 30 minutes and 29.32 O.D./mm^2 and 1034.1 nmol/mg protein/h at 24 h. PRL did not demonstrate any effect on expression and activity of 5'D even at the highest concentration, neither 30 minutes nor 24 hours. Our study showed that in the cow PRL does not contribute to 5'D regulation. This finding demonstrates once more the lesser importance of PRL in cow's mammary gland regulation with respect to rodent's.

References: Aceves *et al.*, 1999, *Endocrinology*, 140, 2948-2953; Kahl *et al.*, 1995, *J. Dairy Sci.*, 78, 2150-2158; Leonard and Rosenberg 1980, *Endocrinology*, 107, 1376-1383. Supported by M.U.R.S.T. 60%

Key words: 5'-deiodinase, prolactin, mammary gland.

P60 **PROGESTERONE (P4) INFLUENCES ON LUTEAL AND ENDOMETRIAL CELL FUNCTIONS THROUGH MEMBRANE BINDING SITES IN COW.** M. Bogacki, W. J. Silvia, J. Mlynarczuk, J. Kotwica; *Institute of Animal Reproduction and Food Res., 10-718 Olsztyn, ul Prawochenskiego5; Poland*

Progesterone (P4) controls proliferation and differentiation of endometrial cells, regulates release of ovarian oxytocin (OT), prostaglandins, (PG) in corpus luteum (CL) and uterus, and inhibits OT-stimulated PGF2α secretion in uterus. These influences can be mediated by cyosolic classical receptors or by lately found membrane binding sites for P4 on cellular membranes in ovary and uterus. The aim of the present study was to evaluate whether: (a) P4 in bovine luteal cells acts through membrane binding sites, and (b) P4 cytosolic/nuclear receptor is necessary for the inhibition of OT-stimulated PGF2α secretion from bovine endometrial epithelial cells. It can be essential for verifying the mechanism of progestin drug action. Exp. 1a. To establish dose of actinomycin D (Act D; inhibits the transcription of DNA to RNA), the luteal cells were incubated with 1, 5, 10 ng/ml of Act D and with or without TNF-α (50 ng/ml). After 24 h medium

was collected for PGE2 measurement. TNF-α increased 8 times secretion of PGE2. Act D alone did not change secretion of PGE2 but each of its dose diminished stimulatory effect of TNF-α. Exp 1b. To establish the importance of nuclear participation in P4 influence on PGE2 secretion, the luteal cells were incubated with LH (100ng/ml) and with P4 (10^{-7}M), either in presence or absence of Act D (1ng/ml). After 24 h of incubation medium was collected for PGE2 and P4 assay. Secretion of PGE2 in luteal cells was increased by P4 ($P \leq 0.05$). Concomitant treatment of luteal cells with P4 and Act D stimulated PGE2 secretion with the similar potency as P4 alone. Neither P4 nor P4 together with Act D changed concentration of PGE_2 in medium. LH increased P4 concentration by 150%, indicating that cells were reactive during entire period of culture. Exp. 2. To study whether P4 inhibits OT effect on PGF2α secretion (without participation of cytosolic/nuclear receptor), the endometrial epithelial cells were incubated in DMEM/Ham's F-12 medium for 7 days with and for 24 h without FCS. Then cells were treated with P4 (10^{-7}M), OT (10^{-7}M), P4/OT with and without Act D (1 ng/ml). Medium was collected after 4 h for PGF2α measurement. Both OT alone and together with Act D stimulated secretion of PGF2α from endometrial epithelial cells. P4 inhibited stimulatory effect of OT. This OT effect was also inhibited by P4 when medium was supplemented with Act D. Exp 3. To study whether P4 action in CL and in endometrium is associated with intracellular Ca2+ mobilization the luteal cells and endometrial epithelial cells were incubated with fura-2 by 1 h. Immediately after treatment with P4, OT and combination of these treatments, the cells were monitored under reverse microscope and changes of intensity of Ca2+ with fura-2 were measured every 10 seconds through 2 minutes. Mobilization of intracellular Ca2+ was observed after P4 treatment in luteal cells and after OT in endometrial epithelial cells. Pretreatmnent of endometrial cells with P4 diminished stimulatory action of OT on Ca2+ mobilization. In conclusion, P4 stimulated PGE2 secretion from luteal cells in non-genomic way because Act D did not prevent this process and P4 is able to mobilized intracellular calcium in short time period. P4 also diminished stimulatory effect of OT on PGF2α secretion without nuclear mediation in endometrial epithelial cells and this process seems to be mediated by cellular membrane binding sites .

Keywords: progesterone, oxytocin,uterus, corpusluteum

P61 **ESTRADIOL REGULATES PRODUCTION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBP) FROM BOVINE GRANULOSA BUT NOT THECAL CELLS.** *L. J. Spicer* and C. S. Chamberlain. Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, 74075 U.S.A.

In cattle, IGFBP-2, IGFBP-4 and IGFBP-5 are predominately localized in large subordinate and small follicles, with little to no activity of these three IGFBPs detected in the follicular fluid of estrogen-active dominant follicles (Echternkamp et al., 1994; Stewart et al., 1996). The varying pattern of these IGFBPs within the follicle during the estrous cycle suggests that IGFBP-2, -4 and –5 are hormonally regulated. In contrast, levels of IGFBP-3 remain constant during folliculogenesis (Echternkamp et al., 1994; Stewart et al., 1996). To determine the effects of estradiol and LH on IGFBP production by granulosa and thecal cells, both cell types were collected and cultured in serum-free medium with various hormone treatments, arranged in three experiments. Following a 2-day plating period and 24-h treatment, cells were enumerated and media were collected, concentrated 10-fold and subjected to ligand blotting. The major forms of IGFBPs produced were a 27-34-kDa IGFBP (IGFBP-2 and –5), and a 20-22-kDa IGFBP (IGFBP-4) by the granulosa cells and a 40-44-kDa IGFBP (IGFBP-3), 34-kDa IGFBP (IGFBP-2), 27-29-kDa IGFBP (IGFBP-5) and a 20-22-kDa IGFBP (IGFBP-4) by the thecal cells. In Experiment 1, 100 ng/ml of insulin stimulated ($P<0.05$) production of IGFBP-2, -4 and -5 by thecal cells, but had no effect ($P>0.10$) on IGFBP-3 production; 100 ng/ml of luteinizing hormone (LH) stimulated ($p<0.05$) production of IGFBP-2, -3, -4 and –5 by thecal cells. In contrast, 500 ng/ml of estradiol had no affect ($P>0.10$) on IGFBP-2, -3, -4 and –5 production by thecal cells of Experiment 1. Production of IGFBP-2/-5 by granulosa cells from small follicles in Experiment 2 was inhibited ($P<0.05$) by insulin, with estradiol and LH not influencing ($P>0.10$) insulin's inhibitory effect; estradiol reduced ($P<0.05$) basal IGFBP-2/-5 production. In Experiment 3, insulin inhibited ($P<0.05$) production of IGFBP-2/-5 by granulosa cells from large follicles whereas estradiol had no effect ($P>0.10$); LH reduced ($P<0.05$) basal IGFBP-2/-5 production. In contrast, insulin (100 ng/ml) alone had no effect ($P>0.10$) on production of IGFBP-4 by small- and large-follicle granulosa cells. Estradiol (500 ng/ml) inhibited ($P<0.05$) IGFBP-4 production by small- and large-follicles granulosa cells, whereas LH (100 ng/ml) had no effect ($P>0.10$) on IGFBP-4 production by small-follicle granulosa cells but decreased ($P<0.05$) IGFBP-4 production by large-follicle granulosa cells. These results suggest that production of IGFBP-2, -4, and -5 by granulosa and thecal cells of cattle are differentially affected by hormonal stimuli. The decrease in IGFBP-4 levels in large estrogen-active dominant follicles of cattle may be due to direct inhibitory action of estradiol and(or) LH on IGFBP-4 production by granulosa cells.

References: Echternkamp et al., 1994, Biol. Reprod. 51:971-981; Stewart et al., 1996, Endocrinology 137:2842-2850.

Key Words: IGFBP, Estradiol, Granulosa cells, Thecal cells.

P62 **MODULATION OF MAMMARY EPITHELIAL CELL RESPONSE TO RETINOIDS.** Cheli F., Rebucci R., Baldi A., *Department of Veterinary Sciences and Technology for Food Safety, University of Milan, I-20133 Milan, Italy*

The retinoids constitute a large family of natural and synthetic compounds with vitamin A activity or structural homology to retinol, with β-carotene in plants and retinyl esters in animal tissues as the two major dietary sources of vitamin A. The ability of cells to uptake and metabolise retinoids are different and cell-type dependent (2). Retinoic acid, as the bioactive form, is involved in cell growth and differentiation and inhibits the proliferation of many mammary carcinoma cell lines and/or maturation of the cells. The pathways by which retinoids achieve these effects are still poorly understood particularly in non-tumorigenic mammary cells, although most of the actions of retinoic acid is due to its ability to alter gene transcription (3). Aim of this study was to study the effects of retinoids and carotenoids on bovine mammary epithelial cells in culture. Cells were incubated with increasing concentrations, ranging from 10^{-9} to 10^{-5} M, of β-carotene, all-trans retinol, all-trans retinoic acid (RA) and N-(4-hydroxyphenyl) retinamide (4-HPR). The effects of FCS and cell density (3 and 1 x 10^5/ml) on the mammary epithelial cell mitogenic response to retinoids were evaluated. Quantification of cell proliferation was made by measuring methyl-^{3}H-thymidine incorporation at 48 h in culture. Results obtained were calculated as % of the controls. Data are presented as means ± S.E. Mean comparisons were done utilizing Duncan's multiple range test with $P<0.05$ selected as the level of significance. β-carotene and retinoids caused a significant growth inhibition ($P<0.05$) of mammary epithelial cells. For β-carotene, retinol and RA the effect was dose dependent ($P<0.05$). The presence of FCS modulated the cell growth response to β-carotene and natural retinoids, with a loss in the dose dependent response. 4-HRP was cytotoxic at 10^{-5} M. The effects of β-carotene, retinol and RA on cell proliferation were dependent upon cell density. At low cell density, β-carotene and retinol were weak stimulators of mammary cell growth. At low cell density, RA was cytotoxic at 10^{-5} M, while at high density that concentration caused only growth inhibition. These data might indicate that several factors modulate the inhibitory and/or toxic effects of retinoids. The presence of FCS in the culture medium, as a consequence of its protein binding capacity, could have dramatic effects on the bio-availability and uptake of carotenoids and natural retinoids. In the absence of serum, higher intracellular concentrations of RA may be reached, which have a deleterious impact on cells (1).

References: Klaassen I. et al., 1999. *Biochim. Biophys. Acta*, 1427:265. Lansink M. et al., 1997 *Eur J. Biochem.*, 247:596. Silveira I.R. and Moreno F.S., 1998. *J. Nutr Biochem.*, 9:446.

Key words: mammary cells, retinoids, carotenoids.

P63 **PLASMA LEPTIN CONCENTRATIONS DURING TRANSITION FROM ANESTRUS AND THROUGHOUT THE ESTROUS CYCLE IN LUSITANO MARES.** J. Robalo Silva[1], G. Ferreira-Dias[1], R. Agrícola[2], J. Alpoim-Moreira[2], M. Barbosa[2] *[1]CIISA, Faculdade de Medicina Veterinária, R. Prof. Cid dos Santos, 1300-477 Lisboa, Portugal. [2] Coudelaria Nacional, Vale de Santarém, Fonte Boa, Portugal*

Recently, research has been focused on the role of leptin in regulation of reproduction in humans, rodents and domestic animals. During transition from seasonal anestrus into the reproductive season, neuroendocrine changes take place, mainly influenced by photoperiod. Since leptin seems to modulate reproductive activity by stimulating GnRH release, this experiment was designed to evaluate the pattern of leptin secretion throughout the transition period, and the estrous cycle in the mare.

Ten Lusitano mares (3 to 15 years old) were kept in an open paddock and fed 2.3Kg/day of mixed grain plus straw and water *ad libitum*. Their body condition throughout the experiment was good. The animals were observed from the Winter Solstice 1999 until June 2000 or until confirmation of normal cyclic activity. Throughout this period the mares were bled and submitted to ultrasonographic observation of the ovaries three times a week. Two mares were already cycling when blood sampling started, three started cycling during winter, another two in the Spring and the last three remained anestrous till the end of May. This information was used for definition of ovarian activity and was the basis for selection of plasma samples for leptin determinations. Blood samples assayed for leptin by RIA (Linco, St. Charles, U.S.A) were selected in order to have samples collected during anestrus, and during the follicular and luteal phases of the estrous cycle. Samples collected close to the time of ovulation were also assayed. Plasma leptin levels showed a marked tendency to increase with the first ovulation of the year (2.92 ± 0.45 ng/ml)(Mean±SEM), compared to anestrous levels (2.24±0.25ng/ml), although the difference did not reach significance. However, the difference was significant ($p\leq0.05$) between anestrous leptin levels and those of cycles that followed the first ovulation, both the follicular and the luteal phases (3.08±0.28ng/ml and 3.22±0.25ng/ml, respectively). When younger mares (≤4 years old) were compared to older mares (≥6 years old), no significant difference on leptin levels at either phase of the estrous cycle was found. Leptin levels in mares that were already cycling when blood sampling started were identical along the

study and similar to those observed when cyclicity started in the other mares. These results show that, in good body condition score mares, leptin secretion increases significantly from anestrus to cycles subsequent to the first ovulation. Leptin concentrations were unrelated to age but increased from the first to subsequent ovulations suggesting that it might be involved in the stimulation of the hypothalamus-pituitary-gonad function during long days. (This work was supported by grant PRAXIS/CVT/11065/98, from FCT, Portugal.)
Key Words: Leptin, Mare, Seasonal Reproduction

P64 FUNCTION AND JUNCTIONAL COMPLEXES OF MAMMARY GLAND IN LATE LACTATING DAIRY GOATS . A. Baldi , S.C.B. Modina*, F. Cheli, L. Baraldi Scesi*, L. Pinotti, F. Gandolfi*. *Dept. of Veterinary Sciences and Technology for Food Safety, *Dept. of Anatomy of Domestic Animals, Faculty of Veterinary Medicine, University of Milan, Italy.*

Lactation progress is characterised by massive changes in the mammary cell population (1). After peak of lactation, in dairy animals, a gradual involution in the mammary gland occurs. During lactation the tight junctions form a highly impermeable barrier between milk and interstitial fluid. It has been demonstrated that intact cellular junctions and in particular intact tight junctions (TJ) prevent paracellular leakage of blood serum into milk and is closely related to the rate of milk secretion. During lactation, several factors such as milk stasis and mastitis can affect epithelial permeability (3). During involution the leakiness of epithelial barrier can be due to the state of the TJ as well as the death of mammary epithelial cells (2). Aim of this study was to verify the integrity of mammary epithelial barrier in dairy goats in late lactation, stimulated or not with GH. We evaluated the changes in milk Na and K as indicators of TJ permeability and somatic cell counts as mammary gland health indicator. We studied the distribution and localisation of caderin-E, desmoplakin and zonula occludens protein 1 (ZO-1), selected as specific molecules of the zonulae adherentes, desmosomes and tight junctions respectively. In addition, we analysed actin microfilaments as cytoskeleton component directly involved in junctional complexes. Six Saanen goats in late lactation, 180 ± 11DIM, were divided into two groups of 3 animals each: one group received 120 mg of slow releasing bovine GH for 4 times at 14 days of interval; the other has been treated with saline (control). At the end of the experimental period, goats were milked out and slaughtered; the mammary glands were removed and processed for indirect immunofluorescence on frozen sections and chemical analysis. Milk production and composition (means ± SEM) of the control and treated goats were respectively: milk production, 1.83 ± 0.33 vs. 2.60 ± 0.33 kg/d; milk fat, 2.74 ± 0.08 vs. 2.46 ± 0.08 %; milk protein, 3.23 ± 0.17 vs. 2.86 ± 0.17 %; SCC, Log $_{10/1000}$ 2.81 ± 0.34 vs. 3.02 ± 0.34. Milk K concentration was significantly higher (48.03 vs. 42.65mM; $P < 0.05$), and the Na/K ratio significantly lower in the treated group (0.31 vs. 0.39; $P < 0.05$). Mammary total DNA was higher ($P < 0.01$) in treated goats than controls (3.23 vs. 2.29 g). Localisation of desmoplakin, ZO-1 and actin, suggests that GH preserved desmosomes and tight junction between secretory cells of functional alveoli but had no evident effect on zonulae adherentes, as indicated by Caderin-E localisation. Considering that mammary glands were processed immediately after complete milking, our data may suggest a better integrity of mammary epithelial barrier in GH treated goats.
Reference: Knight, C. H., 1997. *Livest. Prod. Sci.* **50**, 1. Nguyen, D. D., and M. C. Neville 1998. *J. Mammary Gland Biol. and Neopl.* **3**, 233. Stelwagen et al., 1994. *J. Dairy Sci.* 77, 426.
Key words: goat, mammary gland, GH.

P65 PLASMA, COLOSTRUM AND MILK LEPTIN CONTENT IN DAIRY COWS UNDER CHOLINE SUPPLEMENTATION. Pinotti L., Rosi F.*, A. Baldi. *Department of Veterinary Science and Technology for food Safety, Faculty of Veterinary Medicine, Institute of Animal Husbandry, Faculty of Agronomy - University of Milan, I-20133 Milan Italy.*

In ruminants, plasma leptin is related to changes in body fatness, in nutritional status and in whole body energy balance (1). However when milk leptin is considered, few information are available (3). Results from previous studies showed that leptin was present in human milk and its concentration was related to maternal plasma leptin and adiposity (2). The objective of this study was to determine the leptin content in plasma, colostrum and milk when a lipotropic substance such as choline is supplemented to transition dairy cows. 26 Holstein cows were assigned by parity and average of production in the previous lactation, to one of two groups: control and treated cows receiving 20g of choline in a rumen protected form. Treatment was administered from 14 days before calving through 30 DIM. DMI were measured for each group. Colostrum and milk yield and composition were measured on day 0, 10, 20 and 30 of lactation. Blood was sampled 1 week before calving, and on 0, 10, and 20 DIM. Plasma, colostrum and milk of 10 cows (5 control and 5

treated) were analysed for leptin content using a commercial multi-species leptin RIA kit (Linko Res. Inc., St. Charles, MO). The mean prepartum and postpartum DMI were 11.26 vs. 11.43 kg/d, and 19.41 vs. 19.90 kg/d, for control and treated group respectively. Leptin concentration was increased in colostrum (+128%) than in mature milk, while no differences were observed between control and treated group for both colostrum (13.92 vs. 12.97 µg/l) and milk leptin content (6.11 vs. 5.68 µg/l). Milk leptin levels were higher than those reported in a previous study in lactating cows, in the present experiment we found leptin concentration in mature milk 34% and 49% higher than that reported for cows on day 61 and on day 190 of lactation by Rosi et al. (3). Mean plasma leptin was higher ($P<0.01$) in treated cows (3.19 µg/l vs. 2.34 µg/l) than in control ones. Significant ($P<0.01$) interactions of treatment and time for plasma leptin were observed until calving, while after that only a tendency ($P=0.08$) was found on 20 DMI. Delavaud et al. (1) have indicated in ruminant, that plasma leptin is related to variation in body fatness (35%) and, to a lesser extent, in nutritional status (17%). From our data it is not possible to conclude the same, even if control cows lost BW more rapidly than treated cows after parturition, this difference was not significant. Leptin concentrations in plasma were not correlated with milk leptin content. To conclude, plasma level of leptin was improved by choline supplementation, while the treatment did not affect both colostrum and milk leptin concentration. These results indicate that lipotropic substance administration may affect plasma leptin without substantial effect on milk leptin content.

References: Delavaud et al., 2000, *J. Endocrinology* **165, 519;** Houseknecht et al., 1997, *Bio. Biophysical Res. Comm.* 240, 742; Rosi et al., 1999, Page 422 in *Recent Progress in Animal Production Science*, Ed. by Bertoni et al., Franco Angeli Publ. Milano, Italy.

Key words: leptin, milk, plasma, choline.

P66 **SLAUGHTER-INDUCED HORMONE VARIATIONS.** *D. Gerin*, A. Comin, M. Corazzin, M. Tassinari, A. Prandi. University of Udine, I-33100 Udine

Our aim was to understand if a very stressful event such as slaughter modifies an animal's hormone status and to verify if differences exist between intensively and extensively bred cattle. 18 Pezzata Rossa Italiana bullocks were considered, 7 had been bred and selected in a mountain environment with typical extensive conditions (ES) whereas 13 were specialised in meat production and bred according to substantially intensive techniques (IN). The ES herd consisted of 7 bullocks weighting 560±107Kg (mean±s.d.) at slaughter at the age of 20±9.6months. The IN herd consisted of 13 bullocks weighing 600±19.9 Kg at the age of 20±1.4 months. The ES bullocks were bred at a nutrition level (net energy received/net energy for maintenance) of 1.5, and the IN bullocks of 1.7. Two blood samples were taken from each animal in the farm, on the day before transport to the slaughterhouse, and at jugulation. Special attention was addressed to all animal-handling operations from departure to arrival to the slaughter house, to avoid unwanted stress. The animals were transported for 20 Km in 30 minutes and were slaughtered immediately after arrival in the same facility and under the same conditions. The plasmatic hormones analysed were: IGF-I, hydrocortisone (C), testosterone (T), leptin (L), T3, T4, insulin (I), NEFA in order to control possible metabolic stress. In extensively bred animals, at slaughter there was an increase in C (from 2.3±2.33ng/ml to 17.37±6.21ng/ml, $P<0.01$), in NEFA (from 56±40Ueq/l to 348.29±234.84Ueq/l, $P<0.05$) and a reduction in T (from 6.07±2.45ng/ml to 1.77±1.29ng/ml, $P<0.05$), while there were no variations in IGF-I (from 183±103.07ng/ml to 125.88±96.27ng/ml, $P>0.05$) L (from 2.59±0.44ng/ml to 2.51±0.34ng/ml, $P>0.05$), T3 (from 2.01±0.45nm/l to 1.9±0.52nm/l $P>0.05$), T4 (from 0.04±0.01nm/l to 0.04±0.02nm/l, $P>0.05$) and I (from 6.91±4.9uUI/ml to 7.56±5.99uUI/ml, $P>0.05$). Therefore, in these animals slaughter caused variations in the levels of C, T and NEFA, but such variations were not particularly important (1). In intensively bred animals, there was an increase in C (from 5.6±4.35ng/ml to 32.78±7.56ng/ml, $P<0.01$), and a reduction in T (from 6.86±4.82ng/ml to 3.62±1.78ng/ml, $P<0.05$), whereas no variations occurred in IGF-I (from 283.66±81.16ng/ml to 262.14±77.53ng/ml, $P>0.05$) and L (from 2.79±0.55ng/ml to 2.83±0.53ng/ml, $P>0.05$). These data show that the absolute values of hormone variations at slaughter differ between IN and ES, i.e. it can be noted that C in IN increases in agreement with bibliographic data (1), whereas in ES animals these increases are significantly lower. In general, IN animals, as compared to ES animals, had higher levels of C (5.6±4.35ng/ml against 2.3±2.33ng/ml of ES, $P<0.01$) and IGF-I (283.66±81.16ng/ml as opposed to 122.82±106.93ng/ml of ES, $P<0.01$). Instead, there were no differences in the levels of T (6.86±4.82ng/ml against 6.07±1.77ng/ml of ES, $P<0.05$) and L (2.79±0.55ng/ml against 2.59±0.44ng/ml of ES, $P>0.05$). These differences are probably due to the different type of breeding and to the fact that IN animals are mainly selected for beef production. To conclude, it can therefore be said that extensively bred animals are less "stressed" and, presumably, can thus provide high quality meat (2). Furthermore, in all animals slaughter led to a significant variation in the plasmatic levels of hydrocortisone, NEFA and testosterone, but not of leptin, T3, T4 and insulin.

References: (1) Grandin, 1997, J. Anim. Sci 75:249-257; (2)Scanga et al. 1998, J. Anim. Sci. (76):c 2040-2047

Key words: Hormones, Stress, Slaughter

P67 REPEAT BREEDER COWS AND FOLLICULAR ENVIRONMENT. A. Comin, D. Gerin, V. Marchi, A. Cappa, M. Messina, S. Dal Mas, R. Renaville*, A. Prandi . *Università di Udine, Udine, Italy and *Gembloux Agricultural University, 5030 Gembloux, Belgium*

According to a study at the Provincial Association of Breeders of Vicenza concerning 28,000 milk cows, we noted that before 130 days postpartum, HDR (heat detection rate) and CR (conception rate) were 33% and 40%, respectively. It was also found that 171 days after parturition, 16% of the animals had reproductive problems, either because not pregnant or still to be fecundated. It is known that the prolonged negative energy balance status after delivery influences reproductive efficiency (1) by altering ovarian hormones and follicular development (2). In view of the fact that 171 days after calving the postpartum energetic debt can no longer be a limiting factor (3), we wondered if it would be possible to measure the differences in terms of plasma and follicles. To this end, we tested a well-managed breeding establishment (35 l of milk/cow, 3.5% fat; 3.3 % protein; 119 days PACO (from delivery to conception); 256,000 somatic cells) with pluriparous Italian Friesian dairy cows aged between 3 and 4 years, loose housed and with a regular luteal activity. The selection was based on distance from parturition: 8 had calved at least twenty days before (group A), and 8 at least 150 days before (group B). In both groups, there were no significant differences in NEFA (161 ± 78.25 uEq/l group A; 201.53 ± 123.70 uEq/l group B) and in IGF-I plasmatic levels (44.4 ± 15.34 ng/ml group A; 76.82 ± 50.87 ng/ml group B), thus indicating that the animals had overcome their energetic debt. The ecographic survey effected on follicles every 2 days revealed that 60 days after parturition follicle diameter was substantially smaller ($P< 0.05$) than that of 170 days after parturition. We took a sample of follicular liquid from follicles with diameter greater than 0.8 cm, using an ultrasound guidance probe. All cows in group A had at least one active oestrogen follicle, whereas in group B this was present in only 3 cows out of 8. Plasma levels of leptin were significantly higher in group B cows (2.72 ± 1.02 vs 4.92 ± 1.89 ng/ml) indicating greater stored energy in this group. Likewise, leptin in follicular liquid showed higher concentrations in group B (1.61 ± 0.79 vs 4.57 ± 1.36 ng/ml). One could suppose that animals with a regular luteal activity which did not succeed in being fecundated within 170 days after calving have alterations in their follicular environment preventing them from producing a competent oocyte. It is, therefore, reasonable to think that other factors, independent of energetic debt, can influence the follicular environment.

References: (1) O'Callagan D.O., Boland M.P., 1999, Anim. Sci. 68, 299; (2) Kendrick et al., 1999, J Dairy Sci. 82, 1731; (3) Beam SW and Buttler WR., 1998, J. Dairy Sci. 81, 121.

Key words: cow, repeat breeder, follicle, hormones

P68 ASSESSMENT OF FAECAL CORTISOL METABOLITES IN EGYPTIAN BUFFALO-HEIFERS FOR NON-INVASIVE EVALUATION OF STRESS HORMONES. El-Battawy, K.A.I.; *National Research Centre, Animal Reproduction and Artificial Insemination Dept, Tahrir street Dokki, Egypt.*

It has been demonstrated that a physiological response to stress involves the release of pituitary derived adrenocorticotropic hormone (ACTH) and subsequent secretion of glucocorticoids from the adrenal cortex. Cortisol concentrations in the blood have been widely used to reflect the effects of various stressors in different mammals but blood sampling itself rnay cause stress which consequently interferes with the cortisol values. Therefore non invasive methods fòr determining glucocorticoids or their metabolites are a prerequisite for assessing stress. Above all faecal samples offer the advantage that they can be collected easily without handling the animals. This fact is especially important in buffalo-heifers which are freely reared on the farm. Palme and Möstl (1997) characterised some of the metabolites of infused ^{4}C-cortisol in ruminants, which led to the establishment of an enzyme immunoassay (EIA) for 11,17-dioxoandrostanes. The aim of this study is to evaluate the biological relevance of this EIA in buffalo heifers. Stimulation of the adrenal cortex using ACTH should be tested in buffalo-heifers to investigate the patterns of faecal cortisol metabolites following these administrations.

Keywords: buffalo-heifers, stress, cortisol metabolites, faeces.

P69 MEASUREMENT OF FAECAL PROGESTERONE METABOLITES BY EIA AS AN AID FOR PREGNANCY DIAGNOSIS IN BUFFALOES. El-Battawy, K.A.I, *National Research Centre, Animal Reproduction and A.I Department, Cairo, Egypt.*

The objective of this study was to find out if the concentrations of progesterone metabolites in faeces would reflect the concentrations of progesterone in the blood and consequently be used for non invasive monitorig of the reproductive status in buffalo-heifers. In this investigation, five buffalo-heifers were randomly selected from a group of buffaloes

reared with intact healthy fertile bull. All heifers were rectally examined ten days before and at the start of the experiment. It was proved that three of them were early pregnant while the other two were non pregnant. Blood samples were collected from jugular vein of each animal twice weekly for five weeks to measure plasma progesterone by RIA. On the other hand the faecal samples were collected at the same time as the blood samples to measure faecal progesterone metabolites (20-oxopregnanes) by EIA. The progesterone concentrations in plasma clearly agreed with the concentrations of progesterone metabolites in faeces. So faecal EIA is a valuable technique for pregnancy diagnosis in buffaloes.

Key words: Buffalo , Progesterone , Blood , Faeces.

P70 THE MECHANISM OF THE EFFECT OF PHYSALIS ALKEDENGI ON ESTROUS CYCLE IN THE EWE. H. Khazali and M. Khatery. *University of Tarbiat Modares, Tehran, Iran*

It has been firmly established that *physalis alkedengi* delays estrous cycle in the mammals. The goal of this study was to determine the mechanisrn through which *physalis alkedengi* delays estrous cycle in the ewe. Fourty ewes were randomly assigned to an experiment with a 2x2 factorial design. Ewes received 0, 250, 500 or 1000 rng of *physalis alkedcngi /Kg* BW daily for 45 days. Animals were bled twice a day throughout the experiment. Blood sarnples were kept at 4° C until centrifugation. A saturated sodium citrate solution (40 µl of sodium citrate solution/ml blood) was added to the samples before centrifugation to prevent clotting of plasma during storage. Samples were assayed for estradiol and progesterone by double-antibody RIA. Ovulations were determined by laparoscopy. Data were analysed by split-split plot in time. Feeding 0, 250 and 500 mg of *physalis alkedengi* did not change the mean plasma concentrations of estradiol and progesterone in the animals. Feeding 1000 rng of physalis alkedengi significantly decreased the mean plasma concentrations of estradiol and progesterone in the animals. The result of this study showed that the effect of *physalis alkedcngi* on the estrous cycle is rnediated through the decrease of the mean plasma concentrations of estradiol and progesterone.

Key words: ewe, estrous, physalis alkedengi, mechanism.

P71 T3 AND T4 CONCENTRATIONS IN LIPIZZAN HORSES. *V. Cestnik*, N. Čebulj-Kadunc, M.Kosec, *Veterinary faculty, University of Ljubljana, Gerbiceva 60,1000 Ljubljana, Slovenia*

The thyroid hormone concentrations in horses can be influenced by pregnancy, lactation, time of day, nutrition and glucocorticoid administration (1, 2). Like in several other animals, circulating concentrations of T3 and T4 in horses increase during fetal development, peak immediately post partum, and decline thereafter (2, 3). The aim of our work was to determine the T3 and T4 values in different ages Lipizzan horses. The investigation was performed in Lipica stud farm (Slovenia) in 172 Lipizzan horses of both genders, aged from 17 days to 27 years. The blood samples were collected from jugular vein using double-ended needles and evacuated tubes with-out anticoagulant. Serum T3 and T4 concentrations were measured by RIA commercial kits (Byk-Sangtec Diagnostica) validated for horses. The T3 and T4 values in foals, mares and stallions of different age groups are represented in table 1. Significant differences among various age groups were determined in T3 as well as in T4 concentrations ($P < 0.001$ respectively). Higher T3 and T4 values were determined in stallions than in mares, significantly different from the age of 6 – 7 years on.

Table 1: T3 and T4 values in Lipizzan foals, mares and stallions of different age group

Hormone (nmol/L)	Gender	Age (years) foals	1	2	3	4	5	6 - 7	8-10	11-15	16 -27
T3	mares	1.48	0.72	0.78	0.74	1.18	1.10	0.81	0.73	0.90	0.95
	stallions	1.44	0.78	0.68	0.80	1.16	1.13	1.10	0.89	1.07	1.13
T4	mares	31.78	26.81	27.93	21.87	24.59	19.77	15.53	18.93	16.30	17.01
	stallions	42.53	33.97	31.09	24.93	24.66	21.33	20.74	24.00	24.36	21.28

As reported in other horse breeds (2, 3) the decline of thyroid hormone concentrations parallel to ageing was observed in Lipizzans, which was more expressive for T4 than for T3. The differences between the mares and stallions were also observed (2, 3), which were mostly insignifficant and more expressive for T4 than for T3 as well.

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Key words: horse, lipizzan, thyroxin; triiodothyronine.

P72 EVALUATION OF FOLLICLE STIMULATING HORMONE (FSH), LUTEINIZING HORMONE (LH), GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR (IGF-I) SECRETION IN CATTLE SELECTED FOR INCREASED OVULATION RATE. *S. E. Echternkamp*, USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA.

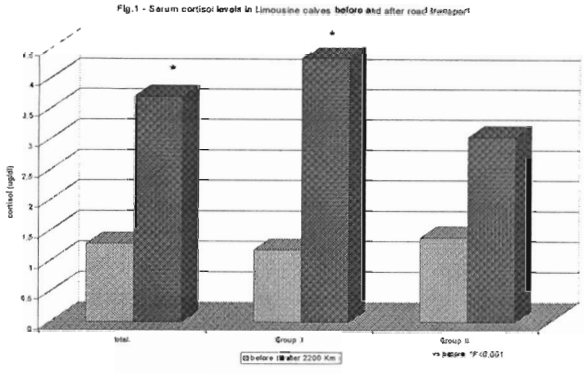
Three generations of genetic selection in cattle for twin ovulations and for dizygotic twin births at the U.S. Meat Animal Research Center has increased the frequency of twin ovulations to 80% and of twin births to 50-55% for the Twinner herd. Ovarian follicular waves of Twinner cows also have a larger pool of small antral follicles (1-5 mm), enhanced recruitment of growing follicles, and the subsequent selection of twin or multiple dominant/ovulatory follicles compared to unselected cattle. The relationship among antral follicular development and FSH, LH, GH, and IGF-I secretion were compared after follicle ablation between cows selected (Twinner) and unselected (Control) for the natural expression of twin/multiple ovulations. Follicular fluid was aspirated transvaginally with an 17-gauge ultrasound-guided needle from all antral follicles >5 mm on both ovaries of 12 Twinner and 12 Control cyclic cows on d 7 or 8 (estrus = d 0). Jugular blood samples were collected at 6-h intervals from 0 to 96 h after aspiration. FSH, LH, GH, IGF-I, and progesterone were measured in plasma by RIA; IGF-I was acid-ethanol extracted. Follicular activity was monitored daily by transrectal ultrasonography. A single injection of prostaglandin F_2 (25 mg, Lutalyse) was administered 4 days after follicle aspiration to facilitate ovulation of the newly formed dominant follicles. Data were analyzed by repeated measures analysis. Plasma FSH concentrations were increased ($P < 0.01$) within 6 h after follicle aspiration, were maximal at 24 h, and returned to baseline by 72 h. Plasma IGF-I concentrations exhibited a small increase ($P < 0.05$) to the aspiration treatment; LH and GH were unaffected ($P > 0.1$) by the aspiration treatment, but GH exhibited diurnal variation ($P < 0.01$). Plasma IGF-I concentrations were greater ($P < 0.01$) in Twinners vs Control cows (56.2 vs 38.1 ng/ml; SEM = 1.8). Plasma FSH (49.9 vs 44.5 ng/ml; SEM = 1.7), LH (1.26 vs 1.06 ng/ml; SEM = 0.04) and GH (6.4 vs 5.3 ng/ml; SEM = 0.7) did not differ between cattle populations. Plasma progesterone concentrations were proportional to number of functional corpora lutea on the ovaries. Ovulation rate was greater ($P < 0.05$) in the Twinner vs Control cows after follicle aspiration. Results suggest that the enhanced recruitment and selection of twin ovulatory follicles is not related to magnitude of concurrent endogenous gonadotropin secretion. However, follicular response to gonadotropins may be enhanced by the greater IGF-I secretion in Twinner females.
Key Words: FSH, IGF-I, Twins, Cattle

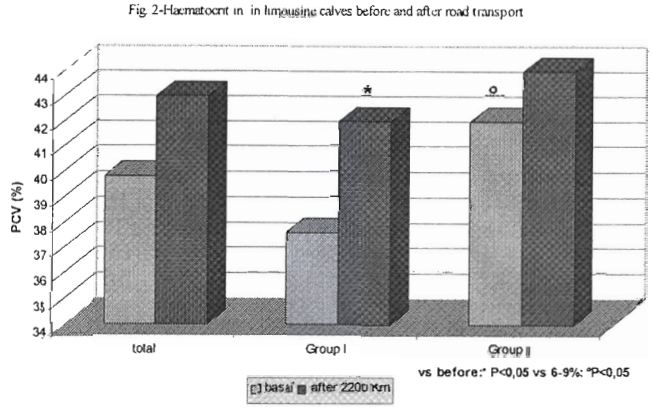
P73 EFFECT OF CHRONIC EXPOSURE TO SELECRONE PESTICIDE ON OVULATION RATE, PROGESTERONE LEVELS AND CHROMOSOMAL ABNORMALITIES IN EWES. *Ahmed S.S.Abdoon*, Wahid M. A and Karima F. Mahrous*. *Department of Animal Reproduction & A.I. and *Department of Cell Biology National Research Centre, Dokki, 12622 Giza, Egypt.*

This experiment was carried out on 13 ewes divided into 2 groups. Group 1 (n=6) received no treatment and served as a control. Group 2 (n=7) was given an oral dose of 0.25 ml Selecrone pesticide for 3 months. After the end of Selecrone administration, both the control and experimental ewes received half norgestomet ear implant for 12 days. At implant removal, 800 IU PMSG were injected i.m. Ewes were monitored for oestrus, timing to oestrus and duration of oestrus was recorded. Ovulation rate was determined on day 6-post oestrus using transabdominal ultrasound scanning and mid-ventral laparotomy. Blood samples were collected before norgestomet application, at PMSG injection, during oestrus and on day 6-post oestrus. Serum progesterone levels were measured using RIA kits. Results indicated that timing to oestrus was significantly longer ($P<0.05$) and number of un-ruptured follicles were significantly greater ($P<0.05$) in Selecrone than control group. Number of CL was significantly greater ($P<0.01$) in control than Selecrone group. Transabdominal ultrasound scanning failed to predict ovulation rate in ewes. Serum progesterone level was significantly higher ($P<0.01$) in Selecrone group during oestrus, while, on day 6-post oestrus it was significantly higher ($P<0.01$) in control than Selecrone group. Cytogenetical analysis revealed that chronic exposure of ewes to Selecrone pesticide significantly increases ($P<0.01$) the incidence of total aberrant cells, chromatid gaps, chromosome gaps and total structural aberrations. Also, the incidence of deletion and fragments and chromosome breaks were significantly increased ($P<0.05$) after Selecrone administration compared with control group. Non reciprocal translocation 1q 2.6-2.10 to 3q terminal parts were found in one control ewe. In conclusion, chronic exposure of ewes to Selecrone pesticide significantly decreased ovulation rate and serum progesterone levels and increased incidence of chromosomal abnormalities.
Key words: Selecrone; ovulation rate; ultrasound; progesterone levels.

P74 EFFECT OF LONG DISTANCE ROAD TRANSPORT ON SERUM CORTISOL AND HAEMATOCRIT IN LIMOUSINE CALVES AND INFLUENCE OF BODY WEIGHT DECREASE. D. Alberghina, P. Medica, E. Fazio, S. Cavaleri*, A. Ferlazzo. *Department of Morphology, Biochemistry, Physiology and Animal Production – Unit of Physiology, Faculty of Veterinary Medicine, University of Messina (Italy)*

The purpose of this study was to evaluate the effect of road transport of long distance length as relevant stressor on cortisol and haematocrit in 10 Limousine calves, aged between 10 and 15 months, which were transported from France to Sicily over a distance of 2200 Km. Blood samples were taken at the morning immediately before loading and at their arrival to Sicily. Serum cortisol and haematocrit values were also evaluated on the basis of different percentage of decrease of transported calves bodyweight (Group I: 6-8.5 %; Group II: 10-12.3%). Results (fig. 1-2) showed a general significant increase after transport of cortisol levels ($P<0.001$); this increase was evident in both groups but was significant only in Group I ($P<0.001$). Concerning haematocrit values, the increase after transport was significant only





in Group I ($P<0.05$). Haematocrit after transport in Group II, although higher than in Group I, was not significantly increased with respect to basal values. This is due to basal values in Group II that were significantly higher than in Group I ($P<0.05$). A significant correlation was found after transport between cortisol and haematocrit in both groups, but negative in Group I ($r=-0.912$; $P<0.05$) and positive in Group II ($r=0.845$; $P<0.05$). These results seem to suggest that the highest percentage of bodyweight decrease is not related with the highest degree of stress. The positive correlation between cortisol and haematocrit after transport in Group II could suggest that the higher decrease of body weight in Group II than in Group I is due to a supplementary dehydration instead of a loss of body mass. The negative correlation between cortisol and haematocrit after transport in Group I could suggest an effect of increased plasma volume induced by stress or of the bland effect of glucorticoids on liquid ritention.

Key Words: cortisol, haematocrit, transport, calves

P75 EFFECTS OF A BETA-ADRENERGIC AGONIST (SALBUTAMOL) ON MEAT QUALITY AND BLOOD FACTORS IN BROILER CHICKENS. Z. Ansari*, and M.J. Zamiri**. **Dept. of Anim. Sci., College of Agricultural, Univ. of Mazandaran, IRAN and **Dept. of Anim. Sci., College of Agricultural, Univ. of Shiraz, IRAN.*

Effects of salbutamol feeding was studied on carcass characteristics and blood parameters in Aryan broiler chickens, from day 30 to day 49 of the rearing period. Salbutamol in solution was fed on 56 chicks per treatment, at the rate of 0 (T_0), 5 (T_1), 10 (T_2) and 15 (T_3) ppm of the diet. Blood samples were collected before the start of salbutamol feeding and again before slauther. Carcass characteristics were evaluated at slaughter. Breast and drumstick meat crude protein, fat and dry matter and several blood parameters were determined. Crude protein was determined by the Kjeldahl method, fat by ether extract and dry matter (water content) by oven drying. Serum parameters (glucose, cholesterol, triglycerides, BUN, CPK and GOT) were determined using commercial kits. Data were analyzed by using the GLM procedure of SAS. The level of significance was set at $P<0.05$. Subcutaneous fat weight was significantly reduced in T_1 (23.1% relative to the control group). Head weight was reduced in T_3, feet weight was increased in T_2, and the weight of liver was greater in T_2 and T_3 as compared with the control group. There was a significant increase in cold and hot carcass weight due to salbutamol feeding. Breast and drumstick fat contents (in dry matter) were significantly reduced

in T_1, T_2 and T_3 but breast and drumstick protein contents (in dry matter) were significantly increased in T_1, T_2 and T_3 Dry matter content of the breast meat was significantly increased. Level of creatine phosphokinase (CPK) was significantly lowered in T_1, but cholesterol level was significantly increased in T_3. Salbutamol did not significantly affect glucose, BUN, triglyceride and GOT levels in blood. The data indicated that salbutamol affects body composition and metabolism of the broiler chickens, similar to other beta-adrenergic agonists.

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Key words: Salbutamol, Broiler, Carcass, Blood

P76 DETECTION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN -2, -3 AND -4 MESSENGER RIBONUCLEIC ACID IN BOVINE OVARIAN FOLLICLES.

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Ovarian insulin-like growth factors (IGF-I and IGF-II) are key regulators of ovarian function, however the actions of these ligands are mediated by their association with a number of insulin-like growth factor binding proteins (IGFBPs). There is considerable species variation in the temporal and spatial gene expression patterns for the various IGFBPs during folliculogensis and it is misleading to extrapolate observations made in mice and rats to farm animal species, especially when differences exist in their patterns of follicle growth. The objective of this study was to determine IGFBP-2, -3 and –4 gene expression in bovine ovarian follicles throughout the first follicle wave of the oestrous cycle. Heifers (n=36) were ovariectomised at 36 h (n=7; emergence), 66 h (n=8; post emergence, pre selection), 84 h (n=13; selection) or 108 h (n=8; dominance) after the onset of oestrus. Heifers ovariectomised at 84 h received either no treatment or were treated with a progesterone-releasing intravaginal device (PRID) and 0.75 mg oestradiol benzoate (ODB) i.m. injection from 12 h after the onset of oestrus until ovariectomy. Messenger RNA expression in granulosa cells (GC) and theca cells (TC) of follicles was determined by in-situ hybridisation. Expression of messenger RNA encoding for IGFBP-3 and –4 were localised in TC, while IGFBP-2 was localised in the granulosa cell layer. Results provide evidence for the localised production of IGFBP-2, -3 and –4 in bovine follicles and indicate differential production of IGFBPs in granulosa and theca cells.

References: Armstrong *et al.*, 1998, *Endocrinol.*. 139, 2146; Lucy *et al.*, 2000, *J. Dairy Sci.* 83, 1635; Yuan *et al.*, 1998, *Domest. Anim. Endocrinol.* 15, 55..

Key words: Bovine, follicles, IGFBP, in-situ hybridisation

P77 INVESTIGATION OF ERYTHROPOIETIN LEVELS IN CATTLE INFECTED WITH THEILERIA.

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Erythropoietin (Epo) is a hormone which is primarily produced by the kidneys in response to hypoxia and stimulates erythropoiesis (1) The aim of this study is to find out whether Epo is stimulated or not in cattle infected with tropical theileria. In this study, serum samples obtained from 12 cattle infected with tropical theileria and 6 healthy cattle were used as materials to detect Epo levels. The disease was diagnosed microscopically in the blood smears of the animals. Microhematocrit values of the animals were also recorded (2,3). Microhematocrit levels were 10.6±2.0 % in infected cattle and 32.3±4.0% in healthy cattle. Serum Epo levels were determined by RIA. Epo levels in infected cattle were 58.00±5.05 mU/mL, and in healty cattle were 22.03±4.04 mU/mL. .In conclusion, statistically significant ($p<0.01$) increases in Epo levels were observed in infected cattle compared to the values obtained from healthy ones. This is because, in theileriosis, infected cells are phagositezed, therefore anaemia develops. Decraese in oxygen transfer capacity causes anoxia. Developed anaemia stimulates the release of Epo from kidneys, and increases its serum concentrations.

References: 1-Giger, 1992 Comp. Cont. Edu. Art., 14(1): 25-34; 2-Hashemi-Feshaeki, 1977, The First Mediter. Conf. Parasitol. İzmir-Turkey; 3-Sahu et al., 1996, Indian Vet. J. 73, 995-997.

Key Words: Theileria, Erythropoietin, Cattle.

P78 USE OF REAL-TIME PCR TO DETERMINE HOMOGENITY OF HEPATIC IGF-I EXPRESSION IN SIMMENTAL STEERS WITH AND WITHOUT REVALOR[7] IMPLANTS. B. A. Crooker, L. S. Ma, and W. J. Weber. *Department of Animal Science, University of Minnesota, St. Paul. USA.*

Although hepatic tissue is generally considered to be quite homogenous, the liver is composed of more than one cell type and gene expression among these cell types is known to vary. Objectives of this study were to determine if IGF-I expression was consistent between hepatic samples obtained by biopsy or at slaughter. Steers received Revalor[7] (implant, N=5) or a sham (control, N=5) on day 0. On day 28, hepatic biopsies were obtained from each steer. On day 28, 29, 30, 31, and 32, one control and one implant steer were slaughtered, the liver removed within 20 minutes, and hepatic samples (n=5) obtained from various locations. All samples were rinsed in sterile saline, immediately frozen in liquid nitrogen, and stored at –80°C until processed. Hepatic RNA was extracted, treated with DNase, cDNA produced, and expression of IGF-I determine by real-time PCR using a GeneAmp7 5700 Sequence Detection System with IGF-I primers (forward 5'-TGCGGGGCTGAGTTGGT-3' and reverse 5'-CCGTGGGCTTGTTGAAATAAA-3'). The reverse primer spanned the junction of exons 3 and 4 which prevented amplification of genomic DNA. Sample and calibrator were corrected for cyclophilin expression and results reported as relative amounts of IGF-I mRNA. Effects of sample type (biopsy, slaughter), treatment (implant, control) and their interaction were assessed using GLM of SAS. Expression of IGF-I did not differ between sample type (biopsy, slaughter; 0.76 and 0.64 0.08, P = 0.31). Expression of IGF-I was greater in implants than controls (0.83 vs 0.57 0.08, P = 0.03). There was no interaction between treatment and sample type (P = 0.66). Expression of IGF-I in control and implanted steers averaged 0.60 vs 0.92 0.11 (a 52% increase) for biopsy samples and 0.54 vs 0.75 0.11 (a 40% increase) for slaughter samples. Relative amounts of IGF-I in biopsy and slaughter samples were highly correlated (r = 0.82, P < 0.01, N=10). In nine of the ten steers, these relative amounts of IGF-I differed by less than 0.18 (correlation: r = 0.94, P < 0.001, N=9). Within a steer, variation of IGF-I expression in slaughter samples was generally quite small. Standard errors of the means ranged from 2 to 16% of individual means and averaged 7.8% across the 10 steers. Results indicate a relatively consistent IGF-I expression in the slaughter samples and suggest hepatic biopsies can provide an accurate estimate of this expression.

Key Words: Liver, Biopsy, mRNA, IGF-I

P79 PLASMA TUMOR NECROSIS FACTOR- IS INCREASED WITH OBESITY IN SHEEP. *J. Daniel*, B. Whitlock, T. Elsasser*, J. Baker, B. Steele, D. Pugh, J. Sartin. *Auburn University, Auburn, AL, USA; *USDA, Beltsville, MD, USA.*

Two studies were designed to examine the relationship between tumor necrosis factor- (TNF) and body fat in sheep. For both studies, last rib fat measurements of 20 ewes were measured using ultrasound. Ewes were divided into fat (fat thickness > 1cm; mean = 1.524 0.029 cm; range 1.14-2.18 cm) and thin (fat thickness < 1cm; mean = 0.254 0.025 cm; range 0.03-0.84 cm) groups and were assigned to either fed or short-term restricted groups for a total of 4 groups (fed, fat; restricted, fat; fed, thin; restricted, thin) with 5 ewes per group. Fed ewes had *ad libitum* access to feed and short-term restricted ewes had all feed removed 48 h prior to the initiation of the experiment. In the first study, subcutaneous fat samples were collected from just above the last rib for detection of TNF and the lipopolysaccharide binding ligand CD-14. Cells staining positive for TNF included infiltrating monocytes, some vascular cells, and adipocytes. TNF-like immunoreactivity in adipocytes was sparse, more pronounced in cells in fed fat and thin ewes than in restricted ewes, and localized to membrane regions between adjacent cells in nucleated regions. CD-14 was minimally observed but present in adipocytes and widely expressed in infiltrating monocytes and epithelial vascular cells among and between adipocytes. In the second study, plasma samples were collected every 6 h for 24 h for determination of plasma concentrations of TNF using RIA. Plasma concentrations of TNF were correlated with fat thickness (r = 0.79533, P = 0.0059) and tended to be correlated with insulin (r = 0.27416, P = 0.0566) in fed ewes. Fat ewes had significantly greater plasma concentration of TNF than thin ewes (0.199 0.001 vs. 0.177 0.001 ng/ml; P = 0.0025) and there was a treatment by body condition interaction (P = 0.0103) such that fed, fat ewes had greater plasma concentrations TNF than fed, thin ewes (0.206 0.001 vs. 0.166 0.001 ng/ml; P = 0.0001), but plasma concentrations of TNF did not differ between restricted, fat and restricted, thin ewes (0.191 0.001 vs. 0.188 0.002; P = 0.7256). These data suggest the presence of TNF-like immunoreactivity that is perhaps more membrane associated and paracrine in function than the lower abundant 17 kD form. In addition, the data suggest that elements within fatty tissue can respond to lipopolysaccharide directly through CD-14 interactions and affect adipocyte function. Moreover, these data indicate that circulating levels of TNF are increased with obesity and may be acting as a signal of body condition.

Key words: Tumor necrosis factor-, fat, sheep

P80 **NALOXONE INCREASES THE MATURATION RATE OF EQUINE OOCYTES ISSUING FROM COMPACT CUMULUS.** *M.E. Dell'Aquila*, M. Albrizio, A. Guaricci, F. Maritato, A. Zarrilli, P. Minoia *University of Bari, I -70010 Valenzano (BA), Italy.*

Reproductive biotechnologies in the horse are slow to progress as compared with other domestic species though their clinical interest. Improving the knowledge on mechanisms regulating meiotic maturation of oocytes could provide significant contribution for in vitro procedures of producing embryos and cloning. In previous studies, we demonstrated that the -opioid receptor gene is expressed in bovine cumulus-oocyte complexes (COCs) and that -endorphins and/or the opioid antagonist Naloxone (Nx) influence the in vitro maturation (IVM) rate with a dose dependent effect (1, 2). In the horse, oocytes recovered with a compact (Cp) cumulus show significantly lower maturation rate that those recovered with an expanded (Exp) cumulus (3), thus significantly impairing the amount of oocytes available for in vitro biotechnologies. In this study, we tested the effects of Nx on the IVM rate (metaphase II with 1st polar body extruded) of equine oocytes recovered with Cp or Exp cumulus and cultured in separate groups. Methods for oocyte recovery and culture and for cumulus and nuclear evaluation were previously described (4). Naloxone was added at the concentrations of 10^{-6}, 10^{-8} and 10^{-10} M. Control oocytes were cultured in the absence of Nx (4 trials, 12 to 20 oocytes, for each condition). The addition of 10^{-10} M Nx significantly improved the maturation rate of equine oocytes recovered with a Cp cumulus (56/80, 70% vs 16/48, 33% for treated and control oocytes respectively; $P<0.001$) whereas no effect was found in the groups of oocytes issuing from Exp cumulus at all examined concentrations. These results, together with the observed expression of the -opioid receptor gene in equine COCs by RT-PCR, provide support to the hypothesis that, in the horse, opioids work as local modulators of the cumulus oocyte-complex and that the addition of Nx could be of interest to improve the efficacy of IVM protocols. Further studies are ongoing on the expression of other opioid receptors and on the effects of Nx on the fertilization rates after intracytoplasmic sperm injection (ICSI) in equine COCs.

References: (1) Albrizio *et al.*, 2000 *Theriogenology*, **53**, 486. (2) Dell'Aquila *et al.*, *Proc ICAR, Stockholm 2-6 July 2000*, **2**, 206. (3) Hinrichs *et al.*, 1993 *Biol Reprod*, **48**, 363-370. (4) Dell'Aquila *et al.*, 2001 *Mol Reprod Dev* (In press).

Key Words: Equine, Oocyte, In vitro maturation, Naloxone.

P81 **Total and free iodothyronines levels before and after short and long distance road transport in Limousine calves.** E.Fazio, D. Alberghina, P. Medica, S. Cavaleri*, A.Ferlazzo. *Department of Morphology, Biochemistry, Physiology and Animal Production – Unit of Physiology, Faculty of Veterinary Medicine, University of Messina (Italy)*

The effects of road transport on calves have been investigated by examination of their behavioural, physiological and pathological responses to many stressors that may be related to the transport. In order to evaluate the incidence of transport stress on thyroid function, total and free iodothyronine levels were measured in 10 Limousine calves, aged between 10 and 15 months that were transported from France to Sicily over a distance of 2200 Km. Blood samples were taken during four different times: at the morning immediately before loading, after short distance transport (45-65 Km.), at their arrival to Sicily after long distance transport (2200 Km) and 15 days after the transport. Serum concentrations of T_3, T_4, fT_3 and fT_4 were analysed by immunoenzymatic assay (Roche Diagnostics, Mannheim). The results (Table 1) showed an increase of iodothyronines levels after short and long distance transport and a decrease to basal values 15 days after the transport.

Table 1: Circulating total and free iodothyronine levels in Limousine calves before and immediately after short and long transport and 15 days after the transport

Time Hormone	Basal values	After short distance transport (45-65 Km)	After long distance transport (2200 Km)	15 days after the transport
T_3 (ng/ml)	2.06 ± 0.26	2.25 ± 0.30	2.39 ± 0.53[a]	1.90 ± 0.34
T_4 (µg/dl)	8.62 ± 1.42	10.38 ± 1.93[a]	12.25 ± 2.66[c]	8.51 ± 0.59
FT_3 (pg/ml)	4.7 ± 0.85	5.42 ± 1.03	6.29 ± 1.95[a]	4.82 ± 0.80
FT_4 (ng/dl)	1.56 ± 0.26	1.75 ± 0.24	2.00 ± 0.50[b]	1.55 ± 0.15

Vs Basal: **a** = $P<0.05$; **b** = $P<0.02$; **c** = $P<0.005$

T_3, fT_3 and fT_4 levels increased slightly after short distance transport and significantly (T_3 and fT_3 $P<0.05$; fT_4 $P<0.02$) after long distance length. T_4 increased significantly after short distance transport ($P<0.05$) and its levels continued to increase, with respect to basal values, after long distance transport ($P<0.005$). A significant positive correlation was found between T_3 and fT_3, T_4 and fT_4, T_3 and T_4 at all times of study ($P<0.01$), between T_4 and fT_3 after short and long distance transport and between T_3 and fT_4 after long distance transport. These results suggest that transport stress

induces an increase of activity of hypothalamus-hypophysis-thyroid axis contemporary with peripheral tissue request, that is evident already after a short distance transport and continues to increase after transport of long distance.
Key Words: iodothyronines, transport, calves

P82 CLINICAL EVALUATION OF THE EFFECTS OF PROSTAGLANDIN F2α USED IN DIFFERENT LEVELS OF LUTEAL ACTIVITIES POSTPARTUM IN MANAGEMENT OF THE DAIRY CATTLE REPRODUCTION PERFORMANCES. N.Fejzic*, J. Ferizbegovic**. *Veterinary faculty Sarajevo**, *Veterinary station Tuzla ***, *Bosnia and Hercegovina.*

Hormonal treatments of dairy cattle in postpartum period as a tool for improvement of reproduction performances are widely recognized as scientifically valid approaches. Many authors investigated the effects of such treatments in different postpartum phases, however results of their investigations are still variable in expected effects. A number of such studies were related with role of external PGF2α in follow up of ovarial activities postpartum and evaluation of optimal way to use it with cattle without presence of any reproductive disorders (Burton and Lean, 1995). Some authors in their studies did not find positive effects of this particular management approach (Gay and Upham, 1994). The objectives of this study were to investigate such a treatment in two different postpartum phases and to try to relate the effects of administration of single dose of synthetic PGF2α with luteal activity postpartum measured by progesterone level in blood samples. From a total number of 175 dairy cattle from commercial farm, HF breed, in all parities, we randomly selected 88 and treated them with a single injection of synthetic PGF2α (500 μg cloprostenol) in period from 16 to 26 (n=43) and from 26 to 42 (n=45) days postpartum. The remaining cattle (87) were used as control and did not receive any hormonal treatment in the same period. All cattle from experimental groups were blood sampled, before treatment, in order to investigate level of progesterone as the measure of luteal activity by RIA diagnostic kits. The cattle from this study were clinically monitored in the next six months and reproductive performances were measured with days open parameter. The descriptive statistics of collected data were analyzed and student t test was used for comparisons between groups. The cattle treated with single injection of synthetic PGF2α in period from 16 to 26 days and from 26 to 42 days postpartum had shorter means for days open 61.74 +/-21.74 and 83.13 +/-25.14 in comparison with 96.09 +/-37.75 days for control group. The results of our study showed positive effects of single administration of PGF2α in postpartum period and we confirmed results of other authors (Pankovski et al., 1995). Investigating the level of progesterone in group of cattle from 16 to 26 days postpartum we found 47 % with level of more than 0.50 ng/ml, and we considered this as an indication of follow up of ovarian activity. Analyzing days open data for cattle in this group we did not find significant differences between cycling and non cycling cattle, what was reason to believe that positive effect of single administration of PGF2α in period from 16 to 26 days postpartum was not result of luteolitical but miometral activity. From another side, analyzing the days open data for cattle received single dose of PGF2α in period from 26 to 42 days, and related those results with level of measured luteal activity, we found significantly shorter means of days open for cattle received treatment during level of luteal activity measured with more than 0.50 ng/ml. This was clinical confirmation of luteolitic activity of PGF2α. In conclusion, we found overall positive effects of single administration of PGF2α postpartum, and we suggest it as a simple and useful tool in order to manage dairy reproductive objectives of farmers and achieving better production performance in dairy herds.
References: Burton and Lean, 1995, *Vet.Rec.*136, Gay and Upham, 1995, *J.Am.Vet. Med.Ass.* 205, Pankowski *et al.*, 1995, *J.Dairy Sci.* 78.
Key Words: PGF2α, progesterone, dairy reproduction

P83 PRELIMINARY STUDY OF MICROVASCULAR DEVELOPMENT AND FUNCTION OF EQUINE CYCLIC CORPUS LUTEUM. G. Ferreira-Dias[1], P. Pinto-Bravo[2], L. Mateus[1], J. Robalo Silva[1], J.A.S. Medeiros[3] *[1]CIISA, Faculdade de Medicina Veterinária, R. Prof. Cid dos Santos, 1300-477 Lisboa, Portugal [2] Escola Superior Agrária de Coimbra, 3040 Bencanta, Coimbra, Portugal [3]Faculdade de Medicina, Universidade de Coimbra, Instituto de Fisiologia, 3049 Coimbra Codex, Portugal*

The corpus luteum (CL) is an ovarian transient endocrine organ that secretes progesterone (P_4), a necessary hormone for implantation. In the mare, a deficient production of P_4 due to a primary luteal insufficiency is associated with early embryonic death. Impaired function of ovarian structures might be related to a deficient vascularization, since the CL formation and its endocrine function are closely dependent on the growth of new capillaries. The objectives of this study were to evaluate angiogenesis in the equine cyclic luteal structures and relate it to luteal endocrinological function.

Luteal tissue and blood were collected during the breeding season at an abattoir from randomly assigned cycling mares. Ovaries and ovarian structures were identified and measured. Stage of the estrous cycle was determined on the basis of ovarian structures and plasma P_4 concentration. Based on structure and P_4 production, luteal tissue was classified as corpus hemorraghicum (n=3); middle luteal phase corpus luteum (n=8); late or regressing corpus luteum (n=2) and non functional corpus luteum (corpus albicans; n=2). Luteal tissue, once removed, was immediately fixed in Carnoy for histology. Blood vessels were marked on histologic sections by histochemical techniques. Vascular density was determined using a computerized image analysis system (CAS, Beckton Dickinson) based on the percentage of total histologic area occupied by vascular lumen. Plasma progesterone concentration was determined by RIA. Microvascular area found for the different luteal structures observed were as follows: 3.08±1.21 (Mean%±SEM) for corpus hemorraghicum; 4.24±1.05 for middle luteal phase corpus luteum; 1.48±0.21 for late or regressing corpus luteum and 2.73±0.64 for corpus albicans. No significant differences in vascular density for any luteal structures evaluated were detected by one-way ANOVA on percentage data subjected to arcsine transformation. With respect to plasma samples obtained at different phases, there was a significant increase in P_4 when both follicular and corpus hemorraghicum phases were compared to either middle or late luteal phases (ANOVA, LSD test, $p \leq 0.05$). So far, these findings might suggest that vascular growth of cyclic luteal structures in the mare is coordinated with development of non vascular tissue. However, these results need to be further confirmed with the use of a larger size sampling.
Key words: Angiogenesis, Corpus luteum, Mare, progesterone. (This work was supported by a Grant from C.I.I.S.A)

P84 **EFFECTS OF GLUTATHIONE ON THE GROWTH PERFORMANCE AND GROWTH HORMONE RECEPTORS ON THE MEMBRANE OF HEPATOCYTES IN LANTANG PIGLETS.** *W L Fu* [*] Q.Y. Jiang [*], Y.T.Zhang [*] B.K. Li [**], J.J. Wang [**]. *Department of Animal Science, South China Agricultural University, Guangzhou 510642, P R. China* [*] *and Dongguan Banling pig farm, Dongguan, 523716 guangdong province, P.R.China* [**].

In order to investigate effects of glutathione on the growth performance and the growth hormone receptors (GHRs) on the membrane of hepatocytes in weaned Lantang piglets. 40 piglets of Lantang breed weaned at 28 days of age were divided into 4 groups, each of 10 piglets. The control group was provided with basal diet. For the other three groups, the diets were added with 25, 50 and 100 mg/kg glutathione respectively. The trial lasted 32 days. Blood samples were taken from the anterior vena cava at 28, 43 and 60 days of age, and the concentrations of insulin-like growth factor-I (IGF-I) in sera were determined by RIA. At the age of 28 and 60 days, liver samples were taken from the left lateral lobe and frozen in liquid nitrogen for membrane preparation, in order to test the growth hormone receptor (Meserole et al., 1984; Breier et al., 1989). The results showed that: (1) The body weight, feed intake and feed efficiency of Lantang piglets were enhanced by the addition of 25-100 mg/kg glutathione in the diets. (2) The addition of glutathione significantly increased the serum levels of glutathione and glutathione peroxidase activities, while the concentrations of IGF-I in sera were fairly increased. (3) The binding capacity and the number of binding sites of GHR on the membrane of hepatocytes increased significantly, with the dose of 100 mg/kg glutathione in diet showing the most significant effects. It was concluded that 25-100 mg/kg glutathione in diet promoted the growth perfomance, the binding capacity and the number of binding sites of GHR on the membrane of hepatocytes in Lantang piglets.
References: Meserole et al., 1984. J Anim. Sci., 59(3):650; Breier et al., 1989. J Endocrinology, 123:2.5.
Key Words: glutathione, growth performance, Lantang piglet.

P85 **EFFECTS OF GLUTATHIONE ON THE GROWTH PERFORMANCE AND THE CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I IN SERA OF LARGE WHITE POST-WEANING PIGLETS.** W.L Fu [*] Y.T. Zhang [*], Q.Y. Jiang [*], B.K. Li [**], J.J. Wang [**]. *Department of Animal Science, South China Agricultural University, Guangzhou 510642, P R. China* [*] *and Dongguan Banling pig farm, Dongguan, 523716 guangdong province, P R.China* [**].

This experiment was carried out to investigate the effects of glutathione on the growth performance of weaned Large White piglets. 48 Large White piglets weaned at 28 days of age were selected and divided into 4 groups, each of 12 piglets. Except for the control group, 25, 50 and 100 mg/kg glutathione were added to the basal diet respectively. The trial lasted for 32days. Blood samples were taken from the anterior vena cava at 28, 43 and 60 days of age and the concentrations of insulin-like growth factor-I (IGF-I) in sera were determined by RIA. At the age of 28 and 60 days, liver samples were taken from the left lateral lobe and frozen in liquid nitrogen for membrane preparation, in order to test the growth hormone receptor (GHR) (Meserole et al., 1984; Breier et al., 1989). The results showed that: (1) Adding 25-100 mg/kg glutathione to the diet could promote the bodyweight and average daily gain (ADG) of Large

White piglets and a significant increase in ADG was observed after 100mg/kg glutathione was added during the period of 43-60 days of age. The feed intake and feed efficiency were, to some extent, increased. (2) The addition of glutathione significantly increased the serum levels of glutathione, glutathione peroxidase activities, and the concentrations of IGF-I in sera. (3) The binding capacity and the number of binding sites of GHR on the membrane of hepatocytes decreased, which seemed to be opposite to the changes of IGF-I in sera. Further trials are needed to elucidate the mechanisms.

References: Meserole et al., 1984. J Anim. Sci., 59(3):650; Breier et al., 1989. J Endocrinology, 123:25.

Key Words: glutathione, growth performance, Large White piglets

P86 **ELISA AND RIA VEGF IN BOVINE FOLLICULAR FLUID.** V. Furlan, A. Comin, M. Messina, A. Prandi . *Università di Udine, Udine, Italy*

VEGF is a vascular growth factor which stimulates the permeability, migration, and proliferative and morphological development of endothelial cells (1). These events are important for the development of the follicle and the corpus luteum. The aim of our study was to establish a VEGF assay in bovine follicular liquid. Since there is no kit specifically designed for bovine VEGF, we used an ELISA kit (made by the R&D System company) which uses human recombinant VEGF165, which presents a homology of 95% with bovine VEGF164 (2). We also specified a RIA assay(prepared in our laboratory) using an antibody against the mouse recombinant VEGF165. The same recombinant was used both as a standard and as a tracer. The mouse recombinant VEGF 165 was chosen since it is able to induce mitotic activity in bovine aortic endothelial cells. The samples of follicular liquid used to test the two assay systems (ELISA and RIA) were taken from eleven dominant bovine follicles (diameter >0.8cm, high E/P4 ratio). The follicles were taken from normally cycling high yielding Italian Friesian, synchronized with double prostaglandin at 11 days. The follicles were collected by transvaginal follicular aspiration. The ELISA test was used following the two protocols indicated in the kit, for plasma and cellular culture. Neither of the two ELISA protocols permitted the evaluation of the concentrations of bovine VEGF. The RIA assay on the other hand was able to discriminate samples of bovine follicular liquid. A parallelism test was performed by serial dilution of the bovine sample. The dilution of the samples runs parallel to the standard curve. The variation within the same test, expressed as the coefficient of variation (CV%), was 8.2%, while the variation between tests was 16.5. The ED 50 was 0.8 ng/ml.

References: (1) Ferrara et al. 1997, Endocr Rev. 18, 4; (2) Ferrara et al. 1992, Endocr. Rev. 13, 18.

Key words: cow, VEGF, follicle, RIA, ELISA

P87 **INFLUENCE OF FEED QUALITY ON RELEASE OF GH AND IGF-I, THE DEVELOPMENT OF MUSCLES AND BONES OF BODY PARTS, THE HISTOCHEMICAL PROPERTIES OF LONGISSIMUS AND PSOAS MAJOR MUSCLES IN WAGYU (JAPANESE BLACK HEIFERS).** *T. Gotoh*, M. Matsuzaki***, T. Etoh, M-A Hattori*, Y. Ono**, H. Iwamoto*. *Kuju Agricultural Research Center, Kyushu University, Kuju Oita 878-0201, Japan, *Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan, **Faculty of Agriculture, Saga University, Saga 849-0903, Japan, ***Kyusyu National Agricultural Experiment Station, Kumamoto 861-1192, Japan*

Nutritional status or the absolute quantity of feed was reported to influence markedly pulsatile release of GH in steers (Breier *et al.*, 1986) . However, when animals are fed freely, how kinds of feed reflect to release of GH is unknown. In 3rd ICFAE, we reported the effect of feed quality in GH pulsatile release. In the present study, to evaluate of feed quality in not only GH release but also IGF-I release, its patterns were compared between the Japanese Black heifers fed freely on concentrate (grain) and roughage (hay). And under the condition of endocrine, we researched the development of the 75 muscles and main bones of body parts, the histochemical properties of Longissimus (LM) and Psoas major muscles (PMM) in their cattle. The animal were divided into 2 groups (3 heifers in each group); one group was freely fed with concentrate, the other was freely with roughage from 5 to 10 months. At 10 months of age, blood samples were obtained with inserted jugular catheters at 15-min intervals for 24 hours. The plasma was assayed for GH and IGF-I by radioimmunoassay. After slaughter, the 75 muscles were removed and weighed without peripheral adipose and tendon. Moreover the main 6 bones of body parts were removed for measuring of their weights, length and thickness. Type-I, IIA and IIB myofibers of LM and PMM were enzyme-histochemically distinguished, the percentage distribution and diameter in each type were measured. Volume percentages of intramuscular crude fat were measured with Soxhlet method. Adipose cell size was observed in the diameter with Oil-red O and Azan staining methods. There were obviously differences in pulsatile patterns of GH release for 24 hours between concentrate-fed and roughage-fed heifers. In roughage –fed heifers, many peaks of GH were observed throughout 24 hours. On the other hand, there were

few peaks of GH in concentrate-fed heifers. The concentration of IGF-I did not significantly differ between concentrate-fed and roughage-fed heifers. Large eighteen muscles located at parts adjacent to trunk in concentrate-fed heifers were significantly larger in weight than those in roughage-fed heifers. In bones, only the weight of *Radius* and *Ulna* of concentrate-fed heifers were significantly larger than those of roughage-fed heifers. Percentage of Type IIA myofibers of LM was significantly larger in group R and conversely Type IIB in group C than each other at 5 % level. Meanwhile percentage of Type I myofibers of PMM was significantly larger in group R and conversely Type IIB in group C than each other at 5 % level. Diameters of intramuscular fat cells were markedly different between 2 groups.

Reference: Breier *et al.*, 1986 *J. Endocr.* **111**, 209.

Key Words: GH, Wagyu, Muscle

P88 EXPRESSION OF LEPTIN RECEPTOR IN SWINE AND BOVINE OVARY. N. Govoni, C. Pezzi, A. Zannoni, P. A. Accorsi Galeati G. *Dipartimento di Morfofisiologia Veterinaria e Produzioni Animali, Università di Bologna, Italy.*

Leptin, a circulating hormone secreted mainly by adipose tissue, is a potential signal that may be involved in connecting fat stores with the reproductive system. Consistent with this hypothesis we have recently demonstrated that plasma and follicular fluid leptin levels are markedly influenced by the levels of nutrition (1). The findings that leptin is present in the follicular fluid and that ovarian leptin receptor is expressed in human, rat, mouse and pig ovary, suggest a possible role of this hormone directly on the ovary. The present study has been designed to analyze the mRNA expression of long isoform of leptin receptor (Ob-Rl) in bovine and swine ovary (granulosa, theca, corpus luteum, oocyte, cumulus cells) by reverse transcriptase-polymerase chain reaction. Ovaries were obtained from swine and dairy cattle after slaughter and transported to the laboratory on ice. The ovaries were processed and granulosa, theca cells, oocyte and cumulus cells isolated as previously described (2). Three categories of corpus luteum were identified by gross morphological criteria: early stage, mid-cycle and regressing stage. Total RNAs were extracted from the tissues using Trizol reagent. The messenger RNA was reverse transcribed into cDNA, then amplified by PCR using specific oligonucleotide primer pairs for leptin receptor of bovine and pig derived from the sequence on GenBank DNA (respectively accession U83512 and AF092422). Results of RT-PCR demonstrated extensive distribution of Ob-Rl RNAm expression in the granulosa and theca cells, cumulus cells, oocyte and corpus luteum in both pig and bovine. We have also observed a lower expression of Ob-Rl RNAm in the late luteal phase than early and middle stages in relation to β-actin expression level. Moreover Ob gene expression was undetectable in the pig ovary as determined by RT-PCR whereas it was detected with Southern Blot using a 40 mer oligo labelled by DIG Oligonucleotide 3'-End Labeling Kit and revealed by chemiluminescent detection. After hybridisation, a specific band was detectable in pig granulosa cells but not in theca cells and corpus luteum. In summary the signaling isoform of leptin receptor was identified in both pig and bovine ovarian cells, therefore these results seem to indicate that the swine and bovine ovary are likely target organs for leptin. Work supported by M.U.R.S.T.

References: (1) Galeati et al., 1:50, 14th ICAR, Stockholm 2-6 July 2000; (2) Mattioli et al., J. Reprod. Fertil. 100: 403-409 (1994).

Key words: leptin receptor , ovary, swine, bovine.

P89 CRESTAR® AND PMSG DON'T AFFECT SYSTEMIC INSULIN-LIKE GROWTH FACTOR-I BUT INDUCE OESTRUS IN POSTPARTUM SUCKLING DROMEDARY FEMALES. Hammadi M[1]., Khorchani T[1]., Moslah M[1]., El-Hatmi H[1]., Chammem M[1]., Portetelle D[2]., R. Renaville[2] [1] *Département des Sciences Animales, IRA, Médenine, 4119 Médenine, Tunisie.*[2] *Unité de Biologie animale et microbiénne, FUSA Gembloux, 13 rue Maréchal Juin B-5030 Gembloux, Belgique.*

In North Africa, camel (*Camelus dromedarius*) is a short seasonal breeder and interval between calving averages 24 months. In the objective to reduce this interval many techniques have been developed (Moslah, 1993, Hammadi, 1995). In this study we report the effect of exogenous hormonal treatment during the postpartum period on the systemic concentration of insulin-like growth factor-I (IGF-I), the induction of oestrus and the rate of conception. Ninety-six dromedary females in late gestation and belonging to 13 private herds in southern Tunisia were chosen to be randomly assigned at 3 to 7 weeks postpartum to 3 treatment groups. Group 1 (n = 33) was treated with Crestar® and injected with PMSG (2000 IU) after removal of the Crestar® implant, group 2 (n= 15) was treated with Crestar® and group 3 (n= 48) was treated with PMSG (2000 IU) only. Blood samples were collected by jugular venipuncture at the end of gestation and on day 0 (before treatment) from all dams and on day 9 (implant removal) from females in group 1. Samples were

centrifuged and plasma was stored at –20°C until analysis of IGF-I and progesterone. After calving, the concentration of progesterone decreased ($P< 0.001$) and that of IGF-I increased ($P<0.01$). They averaged 2.5 ± 1.3 ng/ml and 39.0 ± 11.8 ng/ml vs. 0.5 ± 0.4 ng/ml and 44.9 ± 14.9 ng/ml at the end of gestation and after calving, respectively. At the removal day of implant, the concentration of progesterone was lower than 1 ng/ml and the concentration of IGF-I was statistically not different ($P>0.05$) from the initial concentration (47.9 ± 20.9 ng/ml and 49.7 ± 21.7 ng/ml, respectively). Dams exhibited oestrus 24 to 72 h after removal of implant in group 1 and 2 or injection of PMSG in group 3. The percentage of females in heat was not different ($\chi^2= 3.4$, $P>0.05$) in the three groups and it averaged 64.6%. However, the conception rate was less than 5% in the 3 groups. In conclusion, Crestar® and PMSG don't affect systemic IGF-I but induce oestrus in postpartum suckling dromedary females.

(This research was supported by the I.R.A. (Tunisie), ACSAD (Syrie) and D.G.C.I.(Belgique) cooperation grants).

References Hammadi M. (1995). Actes du séminaire sur l'élevage et l'alimentation du dromadaire. Oct. 1992. Options méditerranéennes, Serie B: n°13, pp: 137-141.

Moslah M. (1993). Actes de l'atelier "peut-on améliorer les performances de reproduction des camelins?", 10-12 Sep. 1992, pp: 225-237.

Key words: Dromedary, Crestar®, PMSG.

P90 EFFECTS OF AVIAN PANCREATIC POLYPEPTIDES ON THE SECRETION OF ALBUMIN AND INSULIN-LIKE GROWTH FACTOR-I FROM CULTURED HEPATOCYTES OF RAT.

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Avian pancreatic polypeptide (APP), which is composed of 36 amino acids and secreted from avian pancreas, has growth-promoting activities for broilers (Kimmel et al., 1975; Dai et al., 2000). This in vitro trial was carried out to observe the effects of APP on the secretion of albumin and insulin-like growth factor-I (IGF-I). Hepatocytes were isolated from 35 day-old SD rat by in situ liver perfusion. The perfusion solution was PBS containing 0.05% collagenase (type II) and the perfusion speed between 2-5 ml/min. The liver was taken out after perfusion and hepatocytes were dispersed by forceps. The hepatocytes were then centrifuged at 200 g for 3 times and inoculated on 24-cell plastic plates at 2×10^5 cells/ml. DMEM was used as the medium. After the hepatocytes attached to the surface of the plate, the cells were divided into 4 groups, and APP at concentrations of 0 mg/L, 2×10^{-5}mg/L, 2×10^{-4}mg/L, and 2×10^{-3}mg/L was added to the medium.. The medium was collected after 72 hours of APP treatment, the concentrations of albumin were determined by auto-chemical analyzer and insulin-like growth factor-I was tested by RIA. The results showed that both albumin and IGF-I levels in the medium were increased after APP was added, with 2×10^{-3}mg/L APP showing the significant effect on albumin secretion and 2×10^{-5}mg/L APP inducing the significant influence on IGF-I concentration. It was concluded that the APP could promote the albumin and IGF-I secretion of cultured rat hepatocytes.

References: *Kimmel et al., 1975. J Bio. Chem., 250(24):9369; Dai et al., 2000. Acta Zoonutrimenta Sinica(China), 12(2):39.*

Key Words: avian pancreas polypeptides, albumin, IGF-I, hepatocyte, rat

P91 STUDIES ON SOME TRACE ELEMENTS AND THYROID HORMONE LEVELS IN CALVES THAT ARE NOT GAINING WEIGHT .

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Several factors a play role in the weight loss or not gaining weight growing animals. Some of them are: insufficient food, trace elements, vitamin and mineral deficiencies, hormonal disturbances especially growth and thyroid hormones. In the present study, 11 local animals with normal appetite aged between 6 and 12 months but not gaining weight according to their breed characters and age brought to the University of Yuzuncu Yil, Faculty of Veterinary Medicine, Department of Internal Diseases Clinic were used as test materials. Six healthy animals of the same age that were at normal weight according to their breed character and age were used as control. Clinically, infection and parasite infestation couldn't be diagnosed. Therefore, especially trace elements and thyroid hormone status , that are known to effect weight gain, were aimed to investigate in their blood. After analysis, mean Zn, Cu, Mn, Mg, T3, T4, FT3, FT4 and TSH levels in the test group were 2.69 ± 0.54 (mg/L), 2.29 ± 0.39 (mg/L), 3.47 ± 0.55 (mg/L), 24.1 ± 2.43 (mg/L), 93.55 ± 9.99 (ng/dl), 2.19 ± 0.41 (µ/dl), 1.78 ± 0.22 (pg/ml), 0.52 ± 0.08 (ng/dl), 0.007 ± 0.003 (mIU/ml) respectively, and in the control group were 4.00 ± 0.73 (mg/L), 3.51 ± 0.53 (mg/L), 2.14 ± 0.75 (mg/L), 21.98 ± 3.29 (mg/L), 116.17 ± 13.53 (ng/dl), 4.74 ± 0.56 (µ/dl), 2.47 ± 0.30 (pg/ml), 1.00 ± 0.11 (ng/dl), 0.004 ± 0.004 (mIU/ml) respectively. Apart

from Mn, Mg and TSH levels, other parameters were lower in the test group. However, T4 and FT4 levels were the only parameters statistically significant ($p<0.01$). As a result, faulty feeding in terms of quality and quantity may cause resorption problems and reduce the benefit obtained from feeding and also can reduce thyroid hormone levels which are known to activate metabolism and growth hormone. Finally, it can affect animals' gaining weight and this may cause important economic losses.

References: 1-Ellenberger, 1989 J. Anim. Sci. 67: 1446-1454; 2- Ramsey, 1999 J. Anim. Sci. 77: 2079-2087; 3-Corrigall, 1976 The Veterinary Record, 13: 396-397.

Key Words: Gaining weight, Thyroid Hormone, Trace Elements, Calf.

P92 **GROWTH HORMONE BINDING SITES IN THE HEN THECAL TISSUE.** I. Yu. Lebedeva, V. A. Lebedev, R. Grossman, T. I. Kuzmina*, N. Parvizi. Institute for Animal Science and Animal Behaviour, 31535 Neustadt, Germany and *Research Institute for Farm Animal Genetics and Breeding, St. Petersburg–Pushkin, 189620 Russia.

Growth hormone (GH) is considered to be a modulator of female reproduction (1). Receptors for GH or their mRNA were detected not only in mammalian ovaries (1), but also in fish ovary (2), suggesting a possible role of this hormone in the regulation of ovarian function in vertebrates. The present investigation was undertaken to disclose and characterize GH binding sites in the hen thecal tissue. Theca crude particulate membrane preparations (CPMP) were obtained from the five largest yellow follicles of domestic hens. Ovine GH (oGH) was used as radioligand in binding assays. Ovine GH had been shown to be an effective competitor for chicken GH when binding to chicken liver membranes (3). Equilibrium dissociation constants (K_D) and binding capacities were determined by Scatchard analysis of saturation curves. Specific binding of 125I-labelled oGH to hen thecal tissue was strongly pH dependent. At pH 7.0 the binding (4.6±0.9 % of total counts) was only significant when no less than 600 µg membrane protein per ml was used. There was a striking ten-fold rise in 125I-labelled oGH binding to CPMP as pH decreased from 6.5 (4.1±0.4 %) to 5.0 (46.1±3.4 %, $P<0.001$) employing 100 µg membrane protein per ml. The treatment of CPMP with 4 M $MgCl_2$ at pH 7.0 did not cause oGH binding to increase considerably. Thus the rise in specific binding did not result from the removal of the endogenously bound hormone at low pH. Moreover , specific binding of oGH to CPMP reached its maximum more quickly and at a lower concentration of membrane protein at pH 5.0 when compared to pH 6.3. The binding was not affected by EDTA elimination of divalent cations from the incubation medium at pH 5.0. Scatchard analysis of binding data revealed a single class of high affinity oGH binding sites in CPMP at both pH 5.0 and pH 6.3, with K_D not differing significantly (1.1±0.3 vs. 2.1±0.7 nM). In contrast, oGH-binding capacity of CPMP at pH 5.0 was almost fifteen times higher than that at pH 6.3 (20.2±3.8 vs. 1.4±0.7 pM per mg protein, $P<0.01$). These findings demonstrate the presence of GH-binding sites in the thecal tissue of large yellow follicles in domestic hens. Apparently, most of these binding sites are latent and can be revealed in vitro at low pH.

References: Hull, Harvey, 2001, *J. Endocrinol.* **168**, 1; Gomez et al., 1999, *J. Reprod. Fertil.* **115**, 275; Krishnan et al., 1989, *Mol. Cell. Endocrinol.* **66**, 125.

Key words: growth hormone, binding sites, theca, chicken

P93 **EFFECTS OF ARGININE ON THE CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I AND CORTISOL IN SERA OF BROILERS UNDER HIGH STOCKING DENSITY.** L. Liu, S. N. Yuan, W. L Fu, L.Yang. *Department of Animal Science, South China Agricultural University, Guangzhou 510642, P R. China*

High stocking density is a detrimental factor to broilers (Elfadil et al.,1996; Sørensen et al., 2000). In order to observe the effects of arginine or glycine on the growth performance and the concentrations of insulin-like growth factor-I and cortisol in blood of broilers, 220 Avian broilers aged 7 days were randomly divided into six groups. Birds in the control group were raised under normal density of 0.1 m^2 / bird and provided with basal diet, while the other groups were kept in high stocking density of 0.05 m^2 / bird and feeded with 0%, 0.25%, 0.5%, 1.0% arginine and 0.5% arginine plus 0.5% glycine in basal diet respectively. The birds were weighted weekly and blood samples were taken at 24, 39, 49 days of age. The concentrations of insulin-like growth factor-I (IGF-I) and cortisol were determined by RIA. At the end of this experiment, 8 birds (4 male, 4 female) from each group were slaughtered and the carcass performance was tested. The results showed that: (1) Under high stocking density, the body weight gain and feed intake of broilers decreased, while the percentage of abdominal fat increased. No significant changes were observed in the weight percentages of muscle; (2) Adding arginine or arginine plus glycine could promote the body weight and feed intake of Avian broilers under high stocking density, and 0.5% arginine added to the diet had the most desirable feed conversion. 1% arginine in the

diet significantly decreased the percentage of abdominal fat; (3) The concentrations of cortisol in sera significantly decreased after 0.5% and 1%arginine or 0.5% arginine plus 0.5% glycine was added in diet, while the levels of IGF-I in sera of the birds increased . It was indicated that the addition of arginine or arginine plus glycine to the diet of Avian broilers could alleviate the stress caused by high density density, with the dose of 0.5% arginine showing the best effects.

References: Elfadil et al.,1996. Avian Dis. 40:546; Sørensen et al., 2000. Poultry Sci. 79:864.

Key Words: arginine, high stocking density, broiler .

P94 PLASMA LEVELS OF LH AND TESTOSTERONE AND OESTROGEN RECEPTOR IMMUNOREACTIVITY OF HYPOPHYSIS IN RAMS AFTER INFUSION OF GENISTEIN INTO THE THIRD VENTRICLE. A. Madej[a], K Romanowicz[b], T Misztal[b], B. Barcikowski[b], Y. Ridderstråle[a], *[a]Department of Animal Physiology, Swedish University of Agricultural Sciences (SLU), Centre for Reproductive Biology in Uppsala (CRU), P.O. Box 7045, S-750 07 Uppsala, Sweden, [b]The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland.*

Phytoestrogens mimic the actions of oestradiol and their affinity to oestrogen receptor beta is substantial (Gustafsson, 1999). We found that centrally administered genistein may increase the secretion of LH and prolactin in ewes during the oestrous cycle (Madej et al., 1999). The aim of the present work was to study the effects of central administration of genistein on plasma levels of LH and testosterone in rams during the breeding season and on ERα immunoreactivity of hypophysis. Nine rams of Lowland Breed were used during October and November. Four of them served as control animals. Infusions into the third brain ventricle were performed with calibrated 1.0-ml gas-tight syringes and a microinjection pump. Control infusions during 4 h were done with Ringer-Locke solution at a flow rate 100 µl/h. The dose of genistein was 15 µg/100 µl/h during 4 h from 12 a.m. Blood samples (3 ml) were collected at 10-min intervals from 8 a.m. to 8 p.m. through a catheter from the jugular vein. The experiment was repeated in February, with central infusions of genistein in four animals and with Ringer-Locke solution in three animals. The animals were slaughtered following morning. The head was removed and perfused with buffered saline followed by 4% paraformaldehyd. The brain was dissected and tissue from the hypophysis was postfixed for 40 h in Bouin's fixative and embedded in paraffin. Sections (4 µm) were processed for immunohistochemical localisation of ERα using a mouse monoclonal antibody against ERα (C-311, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). During the first two hours of infusion of genistein the concentration of testosterone was lower in experimental rams than in rams infused with control solution (0.7±0.3 nmol/l vs 3.2±0.3 nmol/l). The plasma concentrations of LH and testosterone increased during the last hour of the experiment after genistein infusion compared to control infusion (2.1±0.2 µg/l vs. 1.3±0.2 µg/l for LH and 3.1±0.3 nmol/l vs 1.7±0.3 nmol/l for testosterone). Strong labelling for ERα was found in the adenohypohysis in both groups of animals. These results will be further analysed for differences, if any. In conclusion, it seems that centrally administered genistein firstly may decrease the secretion of testosterone in rams and secondly stimulate secretion of both LH and testosterone 3 hours after the infusion was terminated. This work was supported financially by the Swedish Council for Forestry and Agricultural Research.

References: Gustafsson, J.A. 1999. J. Endocrinol. 163, 379; Madej et al., Abstracts of 3rd ESDAR, pp. 72-73.

Key Words: LH, testosterone, ER, genistein

P95 PREPUBERTAL BOARS EXPOSED TO THE ENDOCRINE DISRUPTING CHEMICAL DEHP: LATE EFFECTS ON BLOOD TESTOSTERONE CONCENTRATIONS. Magnusson U,[1] Hultén F[1], Norrgren L[2] and Einarsson S[1]. *[1]Dept of Obstetrics and Gynaecology, [2]Dept of Pathology, Faculty of Veterinary Medicine, Centre for Reproductive Biology in Uppsala (CRU), Sweden*

The detrimental effects exerted by chemicals interfering with reproductive endocrinology, designated endocrine disrupting chemicals (EDC), have been explored in vitro-systems, in lower vertebrates and in laboratory rodents. Studies in other mammals are sparse and extrapolations to other species, including man, have to be made by great care. In the present study we use the young boar as a model for studying effects of exposure by EDC on the young prepubertal male animal. Specifically, we wanted to study the effect of the commonly used plastic softener di-(2-ethylhexyl) phthalate (DEHP), on the blood concentration of testosterone, a hormone essential for male reproduction. With respect to effects of EDC, there are effects that are immediate or that are manifested long time after exposure, or that are seen at both occasions. The present study was designed to address these time-aspects on the exposure-effect relationship. The experiment included 13 prepubertal boars weaned at 5 weeks of age and originating from 4 litters.

The exposure period was 5 weeks, starting one week after weaning. From 3 litters one healthy boar was randomly allocated to each of the three treatment regimes. From the 4th litter, boars were randomly allocated just to the DEHP and C treatment (see below). During the exposure period the boars were twice weekly given an i.m. injection of DEHP, 50mg/kg body weight (DEHP-group), oestradiol, 0.25 mg/kg body weight (OEB-group) and 1 ml peanut oil (C-group). The OEB-group was included as a "positive" control group since most EDCs are reported to be "oestrogenic" and the C-group served as proper control group. Single blood samples were drawn before first exposure, the day after last exposure and at the time of sexual maturation, i.e. at 7.5 month of age. Before and immediately after exposure the blood testosterone concentrations were not significantly different between the groups. In contrast, at 7.5 month of age the concentrations in the DEHP-group were significantly ($p<0.01$) higher than in the OEB- and C-groups. The blood concentrations of oestradiol-17β in the OEB-group were significantly higher ($p<0.05$) than in the DEHP-group and C-group immediately after exposure, whereas at 7.5 month of age there were no differences between the three groups. The boars were slaughtered at 7.5 month of age and no differences ($p>0.05$) in relative testicular weight or in total body weight were seen between the groups. The current data suggest that DEHP may have an androgenic effect in the male pig via induction of increased blood testosterone concentrations. The data also suggest that this effect may be induced by prepubertal exposure, without any immediate effect, and manifested later in the sexually mature animal.
Key words: Endocrine disrupters, testosterone, prepubertal, porcine.

P96 OVARIAN DYNAMICS, SECRETION OF GONADOTROPINS, ESTRADIOL, PROGESTERONE AND IMMUNOREACTIVE (IR-) INHIBIN DURING THE ESTROUS CYCLE IN GOATS. *M. Medan*, K. Taya, G. Watanabe, A. Shalaby*, S. Sharawy* *and K. Sasaki***. *Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan,*Department of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt, **Hitachi, Ltd., Central Research Laboratory, Tokyo 185-8601, Japan.*

Ovarian changes determined by daily transrectal ultrasound and its relationship with FSH, LH, estradiol, progesterone and ir-inhibin were investigated in 6 goats for 2 consecutive interovulatory intervals. Estrous cycles were synchronized using 2 injections of PGF2α analogue 11 days apart. All follicles ≥ 2 mm in diameter and CL were measured daily. A follicular wave was defined as one or more follicles growing to ≥ 5 mm in diameter; the day which the follicles growing to 3 mm in diameter defined as the day of wave emergence and the first wave after ovulation defined as wave 1. The interovulatory interval was 21.3 ± 0.7 days (n=12) and the follicular waves emerged on days – 0.6 ± 0.2, 4.8 ± 0.3, 9.3 ± 0.4 and 12.8 ± 0.4 days for wave 1, wave 2, wave 3 and wave 4 (ovulatory wave), respectively (day = is the day of ovulation). The largest follicle of the ovulatory wave was significantly larger than the largest follicle of the other waves. The CL could be identified ultrasonically on day 3 post ovulation and attained a mean maximum diameter of 12.1 ± 0.3 mm at day 8. Transient increases in plasma concentrations of FSH were detected around the day of follicle wave emergence in all animals. FSH was negatively correlated with inhibin and tended to be negatively correlated with estradiol. These results demonstrated that the predominant follicular wave pattern was 4 waves with ovulation from wave 4 in goats. These results also suggest that there is a close functional coupling between changing FSH concentrations and follicular waves emergence in goats.
Key Words: Ovarian dynamics, Goats, Hormones, Ultrasound

P97 EVALUATION OF THE RESPONSE TO BUSERELIN ADMINISTERED 24 HOURS PRIOR TO ARTIFICIAL INSEMINATION IN SARDA EWE: FOLLICULAR DYNAMICS AFTER OVULATION. M.L. Marongiu, A. Branca *, B. Floris, M. Gallus*. *Università di Sassari, 07100 Sassari, Italy, * Istituto Zootecnico e Caseario per la Sardegna, 07040 Olmedo*, **Italy.**

Failure of fertilization and embryo loss during early gestation are major determinants of reproductive efficiency in Sarda ewe (1). A number of studies have demonstrated that treatment with the GnRH analogue buserelin on day 12 post insemination reduces embryo mortality in cattle and sheep (2, 3, 4). In response to this treatment, large ovarian follicles (LF) and circulating oestrogens are supposed to decrease; the resulting elevation in progesterone secretion, due to the increased number of active corpora lutea (CL), should facilitate embryo implantation. The aim of this research was to investigate the ovarian effect of buserelin administered 24 h prior to A.I. in Sarda ewe. Oestrous cycles of 24 multiparous Sarda ewes were synchronised by 40 mg FGA soaked vaginal sponges left in place for 14 d. All ewes received i.m. 400 IU/head PMSG at sponge removal and after 55 h were submitted to A.I. with fresh semen at a dose of 400 x 10^6 spz/head (Day 0). 24 h prior to A.I. (Day –1) 12 ewes (Group T) were given a single i.m. injection of 4 μg/head of buserelin (Receptal, Hoechst Italia, Milan) and the remaining 12 ewes (Group C) acted as control by

receiving sterile saline. Ovulation rate and LF number were assessed by endoscopy on Day 5 and 12 after A.I. On Day 5 a total of 18 CL were observed in Group T vs 12 CL in Group C; 6 LF (∅ ≥ 4 mm) were detected both in Group T and C. On Day 12 a total of 19 CL were found in Group T vs 12 CL in Group C; 3 and 7 LF were detected in Group T and C respectively. All the differences were not significant. In comparison with a previous study (5) in which buserelin has been administered to Sarda ewes on Day 8 or 12 after A.I., we observed a higher number of CL in T subjects. Thus, the timing of GnRH agonist administration seems to influence the degree of ovarian response. Although the greater incidence of ovulations in Group T, only 3 subjects within each group were found pregnant by Day 40 (pregnancy detection by ultrasonography). This was probably related to the fact that the experiment was carried out during the non-breeding season (March). Results from the present study suggest that the buserelin treatment 24 h prior to A.I. in ewes influenced follicular dynamics after ovulation by inducing an increase in the number of CL and a decrease in the number of LF.

References: 1) Branca et al., 1999, *Renc. Rech. Ruminants*, 6, 222; 2) Peters et al., 1992, *J. Phys. Pharm.*, 43 (Suppl. 1), 143; 3) Beck et al., 1994, *Anim. Prod.*, 58, 243; 4) Beck et al., 1996, *Anim. Sci.*, 63, 407; 5) Branca et al., 2001, *Atti So.Fi.Vet.*, 4, in print.

Key Words: Buserelin, Ewe, Corpora lutea, Large follicles.

P98 REPRODUCTIVE EFFICIENCY, PRODUCTION AND GENETIC POLYMORPHISM IN A MOUNTAIN HERD. M. Messina, A.Comin, D. Gerin, R. Renaville *, A. Prandi. *University of Udine, I-33100 Udine, Italy*Gembloux Agricultural University, B-5030 Gembloux, Belgium*

The aim of this work was to evaluate the relationship between reproduction and production efficiency and polymorphism of four selected genes (GH, Pit-1, κ-casein and ß-lactoglobulin) in Pezzata Rossa Italiana cows, selected for both meat and milk. The reproductive efficiency of the tested herd (n=140), located in a mountain environment, was evaluated according to the method devised by Prandi *et al.* (1). Using different PCR methods, the polymorphism of GH (mutation Leu/Val^{127} [alleles A/B] (2), Pit-1 [alleles A/B] (3), k-casein (k-cn) [alleles A,B,C] and ß-lattoglobulin (ß-lg) [alleles A/B] genes were determined. The herd has a good reproductive efficiency with barycenter co-ordinates x=77 and y=35 and the mean value from delivery to parturition of 97 ± 43 days (avg±sd). Perhaps the herd's good reproductive efficiency is due to attentive, accurate management of the reproductive sphere coupled to selection of animals most suited to the mountain environment. In addition, the practice of transhumance compels breeders to fertilise the cows from January to February. Breeders are not interested in fecundating the cows beyond this date, and the animals thus remain unproductive for a long period. Moreover, it is preferred not to give priority to production to the benefit of reproductive efficiency. To this end, breeders make use of natural rather than artificial covering. The allele frequency for the studied genes are illustrated on table 1.

Table 1. Allele and genotype frequency of the selected genes

	Allele A	Allele B	Allele C	Genotype AA	Genotype AB	Genotype BB	Genotype AC	Genotype BC
GH	75,0%	15,0%	-	55,3%	39,3%	5,4%	-	-
Pit-1	13,1%	86,9%	-	-	19,2%	80,7%	-	-
k-cn	52,0%	43,8%	4,2%	22,3%	52,8%	18,5%	5,7%	0,6%
b-lg	68,0%	32,0%	-	49,3%	37,3%	13,4%	-	-

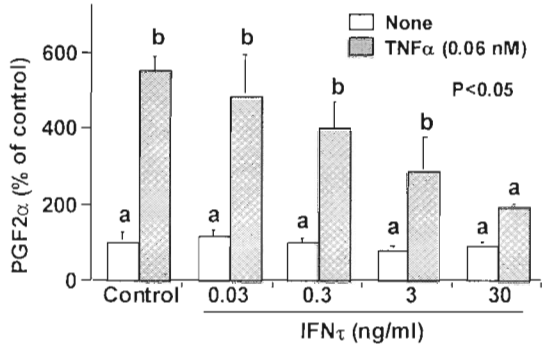
Milk production of the herd is about 40.00 q with 3.9% of fat and 3.25% of protein, as the national average values are respectively 58.6q, 3.89% and 3.4%. The lower frequency of Pit-1 allele A must be associated with the poor milk yield capacity. Difference in milk production of the animals with Pit-1 genotype AB and with Pit-1 genotype BB is statistically not significant. As for milk quality and particularly cheese production, the K-cn and ß-lg allele B could be increased easily by selection. The analysis underlines the fact that in particularly disadvantaged environments, it is considered more important to select animals according to their reproductive efficiency rather than to follow the typical practice of the valleys where, in intensive breeding, it is preferred to select the animals according to their production capacities.

References: (1) Prandi *et al.*,1994, Theriogenology, 42, 65; (2) Parmentier *et al.*, 1999, Dom. Anim. Endocrinol. 17, 139; (3) Renaville *et al.*,1997, J. Dairy Sci., 80, 3431 .

Key Words: P4, reproduction efficiency, production, genetic polymorphism

P99 **INTERFERON-τ SUPPRESSES SECRETION OF PROSTAGLANDINS INDUCED BY TUMOR NECROSIS FACTOR-α IN BOVINE ENDOMETRIAL STROMAL CELLS.** Y. Miyamoto, Y. Kasahara, K. Okuda. *Laboratory of Reproductive Endocrinology, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan.*

Tumor necrosis factor-α (TNFα) has been shown to be a potent stimulator of secretion of prostaglandins (PGs) in bovine endometrial stromal cells (1). Moreover, it has recently been proposed that TNFα is involved in the initiation of luteolysis by regulating the PG secretion from the endometrium in cattle (2, 3). On the other hand, interferon-τ (IFNτ) is known as an embryonic signal responsible for the recognition of pregnancy in ruminants. In the present study, we attempted to determine whether IFNτ modifies the effect of TNFα on PG synthesis by cultured bovine endometrial stromal cells. Cells were obtained from cows at Day 2-5 of the estrous cycle and cultured in DMEM/Ham's F-12 medium supplemented with 10% calf serum. When the cells were confluent (6-7 days after the start of culture), the



medium was replaced with fresh DMEM/Ham's F-12 medium supplemented with 0.1% BSA. The cells were then exposed to TNFα (0.006-0.6 nM) and/or IFNτ (0.03-30 ng/ml) for 24 h. TNFα resulted in increases of PGF2α and PGE2 production by the stromal cells in a dose-dependent manner ($P<0.05$). IFNτ alone showed no significant effect on PG secretion in the stromal cells. However, IFNτ reduced TNFα-induced PGF2α and PGE2 synthesis in a dose-dependent manner ($P<0.05$). When the stromal cells were exposed to TNFα with 30 ng/ml IFNτ, the action of TNFα was completely stopped. The present and previous results (1-3) lead us to hypothesize that IFNτ has a luteoprotective action by inhibiting TNFα-induced PG production from the bovine endometrium.

References: 1. Miyamoto et al. 2000, *Biol. Reprod.* **62**, 1109; 2. Skarzynski et al. 2000, *Biol. Reprod.* **62**, 1116; 3. Murakami et al. 2001, *Theriogenology* **55**, 1667.

Key Words: TNFα, IFNτ, Prostaglandin, Endometrium

P100 **DEVELOPMENT OF ESTRADIOL POSITIVE FEEDBACK ON LH SECRETION IN PREPUBERTAL HEIFERS.** K. Nakada, Y Tanaka, M. Moriyoshi, Y. Sawamukai. *Department of Veterinary Obstetrics and Gynecology, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan*

At puberty, the reproductive system including the hypothalamus-pituitary-gonad axis acquires the periodicity like the sexual matured animals, and the first ovulation occurs in female. At this time, hypothalamus and anterior pituitary gland can respond to evoke preovulatory GnRH-LH surge on circulating peripheral estradiol. Therefore, the positive feedback of estradiol on preovulatory LH surge has to be established before the onset of puberty. Nevertheless, there is little detail information about the establishment of estradiol positive feedback to LH surgical release in prepubertal heifers. Then, our study was carried out to investigate changes in gonadotrophin release to estradiol or GnRH treatment, and to clarify the change of the positive feedback of estradiol in prepubertal heifers. Four prepubertal Holstein-Friesian heifers were used for estradiol treatment study (Exp. 1). The heifers were treated 2 ug/kg estradiol benzoate intramuscularly at 1, 3, 5, 7 and 9 months of age, and plasma samples were collected every 3 hour for 36 hours after estradiol treatment. Fifty prepubertal Holstein-Friesian heifers were used for GnRH treatment study (Exp. 2). Fifty heifers were divided into 5 groups of the one group 10 heifers (1, 2, 4, 6 and 8 months of age). The heifers were treated 1 ug/kg GnRH intravenously, and plasma samples were collected every 30 to 60 min for 360 min after GnRH treatment. Plasma samples in both studies were measured concentrations of LH, FSH and estradiol by RIA. In Exp. 1, the changes in peripheral estradiol concentration after estradiol treatment were similar pattern in all age. LH and FSH rises induced by estradiol were observed in heifers after 3 months of age, and the time from the estradiol administration to the appearance of the peak of gonadotrophin

rise gradually shortened with the age. The peak concentration of LH rise induced by estradiol increased, while the concentration of FSH rise decreased with age. In Exp. 2, the administration of GnRH caused LH and FSH rise with the peak at 30 min in heifers from 1 month of age. The peak LH concentration and the area under the curve (AUC) as a marker for the total amount of LH release increased, while the peak concentration and AUC of FSH decreased with age. These results indicate that (1) hypothalamus and pituitary gland have already had the reactivity for estradiol and GnRH in heifers by the 3 month of age, that (2) estradiol positive effects, including GnRH responsibility in pituitary gland, to LH surgical release develop with age, and that (3) the suppression mechanism on the FSH secretion in pituitary develops with age in prepubertal heifers. We conclude that the development of estradiol positive feedback to LH surgical release before puberty may be one of the essential factors for deciding the time of the onset of puberty in heifers. [This work was partially supported by a Grants-in-Aid to Cooperative Research from Rakuno Gakuen University 2001-6.]
Key Words: Estradiol, LH, Positive feedback, Puberty

P101 **LAMB RESPONSES TO OPEN FIELD TESTING.** *F. Napolitano, A. Braghieri, G. De Rosa*, A. Girolami, M. Albenzio**., A. Sevi**. Dipartimento di Scienze delle Produzioni Animali, Università degli Studi della Basilicata, Potenza. *Dipartimento di Scienze Zootecniche e Ispezione degli Alimenti, Università degli Studi di Napoli Federico II, Napoli, **Istituto di Produzioni e Preparazioni Alimentari, Università degli Studi di Foggia, Foggia, Italy.*

Artificial rearing represents for lambs a combination of emotional (separation from the ewe) and nutritional discomfort (transition from maternal to commercial milk). Isolation tests have been widely used to evaluate adrenal and behavioural responses to acute stress in ruminants. In a series of 4 experiments, a total of 6 open field tests was conducted to evaluate how artificially reared and control (ewe-reared) animals cope with isolation conditions at different ages (10-20 vs. 40-45). Each animal was exposed to a novel environment (a 5 x 4m pen) and isolated from tactile and visual contact with conspecifics for 15 min. However, lambs were able to perceive auditory and olfactory stimuli from other animals. Latency time to the first movement, duration of movement, number of bleats and flight attempts were recorded. For each test, at least 3 blood samples were collected (immediately before isolation, 15 and 45 or 60 min after). Hormone concentration was determined by a RIA specific for ovine cortisol (Dia Sorin, USA). In all experiments isolated subjects displayed the highest cortisol level in samples taken at 15 min (50-60 μg/dl; $P<0.001$), whereas hormone concentration decreased to basal levels at 45-60 min post separation (10 μg/dl). Therefore, all lambs were fully capable of producing an adrenal response to the emotional stress induced by removal from their home pens, isolation, exposure to a novel environment and handling. Younger and artificially reared lambs showed increased levels of plasma cortisol ($P<0.05$). Younger animals are still largely dependent on mothers (Napolitano et al., 1995) and maternal deprivation determines a higher cortisol response in these animals, whereas in older and more independent animals a lower perception of the stress of separation from the group may reduce differences between treatments. When exposed to a novel environment younger and artificially reared animals exhibited withdrawal behaviour. They were slow to initiate movement ($P<0.01$-0.001) and displayed little ambulatory behaviour ($P<0.01$-0.001). Flight attempts were higher in older ewe-reared animals ($P<0.01$), whereas we observed an increased vocal response of younger ewe-reared lambs ($P<0.001$). Both behaviours are likely to be performed by animals motivated to rejoining cospecifics. However, isolation represents a strong emotional stress for gregarious animals like lambs and, as observed for cortisol levels, their behavioural response is age-related. Vocal communication plays a role in maintaining ewe and lambs in contact. However, auditory signals represent a sort of passive behaviour of "calling to be found", therefore suitable for less active animals, such as younger subjects. Conversely, a higher frequency of active behaviours (e.g. flight attempts) has been reported in lambs which are older and, therefore, closer to the weaning age. The response of animals to an overimposed stimulus is likely to be the result of a combination between different motivational systems competing for animal behaviour control. De Passillé et al. (1995) classified the behaviours recorded while animals were tested in an open field according to the motivations that might underlie each response. These authors described three main clusters (fear, exploration and locomotion). Vocalisation was included among variables indicating fear, whereas ambulatory behaviours were associated with locomotory motivation. In particular, the locomotive activity of an animal in a novel situation is likely to be the result of at least two tendencies, the need to explore a novel environment to locate cover, feed, etc. and the tendency to freeze to avoid predators. In this study, ewe-reared subjects, displaying higher levels of ambulatory behaviour, possibly expressed uninhibited exploration rather than attempts to find a way out, whereas, freezing rather than escape may be the predominant response to fear in lambs. Therefore, for these animals a further useful indicator of stress may be latency time to the first movement with long latency being associated with high level of fear However, the interpretation of animal responses to stress is not simple and a higher understanding of the causal mechanisms underlying such responses is needed.

References: De Passillé et al., 1995, Appl. Anim. Behav. Sci. **45**, 201. Napolitano et al., 1995, Appl. Anim. Behav. Sci. **45**, 245.
Key words: lamb, open field test, cortisol, behaviour.

P102 ADRENOCORTICOTROPIN (ACTH) SECRETION BY PORCINE PERIPHERAL LYMPHOCYTES DURING PREGNANCY AND EARLY POST-PARTUM PERIOD. J.B. Phogat, N. Parvizi. Institute *of Animal Science and Animal Behaviour (FAL), Mariensee, 31535-Neustadt, Germany.*

We (1) have recently reported that lymphocytes harvested from pregnant cows secrete higher ACTH than non-pregnant cyclic and cystic cows. The increment in the ACTH release is evident around days 7-10 of pregnancy lasting through out the pregnancy. The present study was conducted to confirm whether ACTH production from swine lymphocytes is also enhanced during pregnancy and early post-partum period. Whole blood (100 ml) was collected via jugular venipuncture from primiparous pregnant sows at days 50 (n=8), 80 (n=9), and 100 (n=7) of pregnancy, and subsequently at days 2 (n=8) and 7 (n=8) post-partum. Similarly, blood was also collected from age-matching non-pregnant sows (n=11). Peripheral lymphocytes were harvested by density gradient centrifugation technique and washed with Hank's Balanced Salt Solution (HBBS) before culture. Lymphocytes were cultured in four well plates with Dulbecco's Modified Eagle Medium for 72 hours at 37C and 5% CO_2 comprising 2 million cells per well. After 72 hours incubation, medium was collected in tubes and stored at –20C until assayed. Adrenocorticotropin was measured by an immunoradiometric assay. In comparison to non-pregnant sows, lymphocytes harvested during pregnancy and early post-partum period secreted higher ACTH i.e. 25-43% ($p \leq 0.01$) and 47-52% ($p<0.001$), respectively. Following delivery (at day 2 post-partum), ACTH secretion further increased and was significantly higher ($p<0.05$) when compared to pregnant sows. However, at day 7 post-partum ACTH secretion was marginally but not significantly higher than during pregnancy. Stimulation of lymphocytes in culture with plant lectin phytohaemoagglutinin (PHA-M; 10 micro gm/well) resulted in an augmentation of ACTH secretion from lymphocytes of non-pregnant sows ($p<0.05$). However, PHA-M did not influence the ACTH secretion from lymphocytes of pregnant and post-partum sows. Results of this study in sows coroborate our earlier findings in cows and suggest that ACTH secreted from lymphocytes during pregnancy might be having some immunomodulatory effect via autocrine and/or paracrine mechanisms. The higher ACTH secretion during post-partum period indicates that other physiological states like stress of parturition, lactation, suckling or uterine involution could be involved in influencing the lymphocyte activity.

References: Dixit and Parvizi, 2001, *Biol. Reprod.* 64, 242.
Key Words: Lymphocytes, ACTH, Pregnancy

P103 INFLUENCE OF TAIL DOCKING, TOOTH RESECTION AND CASTRATION ON PLASMA CORTISOL, ACTH, GLUCOSE AND LACTATE IN PIGLETS. *A. Prunier*, A.M. Mounier, A. Bergeon, M. Hay*. *Unité Mixte de Recherche sur le Veau et le Porc, I.N.R.A., 35590 Saint-Gilles, France and *Ecole Nationale Vétérinaire, 31076 Toulouse cedex 3, France.*

Changes in the activity of the sympathetic nervous system or in the hypothalamo-pituitary-adrenal axis have been extensively used to evaluate pain induced by castration or tail docking in sheep and calves (Molony & Kent, 1997). Such data are missing in pigs. Therefore, 3 experiments were runned to determine the effects of tail docking, tooth resection and castration on plasma cortisol and ACTH. Glucose and lactate were also measured since catecholamines are known to stimulate mobilization of glycogen which results in glucose and lactate release. In Exp. 1 and 2, piglets were catheterized at birth (catheter inserted non-surgically into one umbilical artery under general anaesthesia) and used for the experiment the following day. In Exp. 1, piglets from 7 litters were submitted to one of the following treatments: tail docking, tail docking + a cold analgesic spray, control handling, control handling + spray, no handling (n = 5/group). Tail was docked with an iron docking (cautery) as usually performed in commercial piggeries. In Exp 2., piglets from 9 litters were submitted either to: teeth clipping with pliers, teeth resection with a grinder apparatus, control handling, no handling (n = 6/group). In Exp.3, 17 piglets were catheterized surgically into one jugular vein under general anaesthesia at 5 or 6 days of age. Two days later, they were submitted either to: bilateral castration, control handling, no handling (n = 5 or 6/group). Castration was performed with a scalpel as already described by McGlone and Hellman (1988). Serial blood samplings (1 to 2 ml/sample) were collected before (-15 and -1 min) and after (+5, +15, +30, + 60, +90, +180 min) the experimental treatment. Tail docking with or without the cold spray as well as tooth resection had no marked effects on the patterns of plasma cortisol, ACTH, glucose and lactate (Table 1). Contrarily, castration induced significant increases in ACTH (from +5 to 60 min), cortisol (from +15 to 90 min) and lactate (from

+5 to 30 min). Lack of clear effect of tooth resection and tail docking on plasma parameters may be due to the fact that (a) nociceptive stimuli are not sufficient to elicit a clear answer of the adrenal and sympathetic axes and/or (b) that systems are not fully responsive to stressful event in 1-day old piglets. Measurements of plasma cortisol, ACTH and lactate after castration could be very useful for validating protocols designed to reduce pain induced by castration in 1-week or older piglets.

Tableau 1. P values for each main effect. Data were analysed with the GLM procedure of the SAS package using a split-plot model.

	Tooth resection			Tail docking			Castration		
	Treatment	Time	Treat. x time	**Treatment**	Time	Treat. x time	**Treatment**	Time	Treat. x time
ACTH	**0.85**	0.0001	0.57	**0.12**	0.15	0.014	**0.018**	0.0001	0.0001
Cortisol	**0.83**	0.005	0.97	**0.46**	0.082	0.74	**0.007**	0.0001	0.0001
Glucose	**0.68**	0.70	0.58	**0.12**	0.035	0.77	0.82	0.19	0.59
Lactate	**0.46**	0.0001	0.82	**0.38**	0.0001	0.051	**0.007**	0.0001	0.0001

Key words: piglets, hormones, castration, tail docking, tooth resection

P104 EFFECT OF THE EPIDURAL ADMINISTRATION OF A GnRH ANALOGUE ON LH AND PROGESTERONE PLASMA CONCENTRATION IN CATTLE. A. Quaranta, R. Minoia, P. D'Amico, R.L. Sciorsci. *University of Bari, I-70010 Valenzano (BA), Italy.*

The common route of administration of GnRH and its synthetic analogues is intramuscolar or intravenous. The aim of this work was to assess the effectiveness of the epidural administration of a GnRH analogue in cattle. The estrus of twenty cyclic cows was synchronized using norgestomet and estradiol valerate (Crestar®) followed by cloprostenol (Estrumate®) on day 9. Pituitary function was determined by detecting the preovulatory LH peak. After inducing a second synchronization with cloprostenol, 50 g of lecirelin acetate (Dalmarelin®) were injected in ten animals via the epidural route and in other ten intravenously 15 hours after the first signs of estrus. All animals underwent artificial insemination immediately afterwards. LH was measured every 30 minutes for 6 hours and progesterone every 48 hours for 22 days. LH and progesterone were determined by ELISA and RIA, respectively. Hypophyseal response to lecirelin stimulation was assessed on the basis of the area under the curve (AUC) for LH during the 6 hours after stimulation, the highest blood concentration of LH and the time of its appearance (1). The data were estimated by ANOVA. Results show that both administration routes are able to induce a marked hypophyseal response, and that epidural administration is as effective as the intravenous one, with no statistically significant differences, as shown in the table (mean value ± SEM):

	LH AUC	LH peak	Appearance of LH peak
Epidural	123.4 ± 33.7 ng/ml x 6 h	20.4 ± 6.9 ng/ml	115.2 ± 20.1 min
Intravenous	153.1 ± 68.7 ng/ml x 6 h	26.8 ± 13.0 ng/ml	120 ± 32.8 min

The fertility index was higher in the epidural group than in the intravenous one (70% *vs* 40%), and so was progesterone secretion, (45.1±3.3 *vs* 39.3±10.5 ng/ml), although no statistically significant differences were attained. These results depict a trend, which may be confirmed by testing a larger number of animals. In conclusion, the epidural administration of lecirelin acetate is effective, easy to perform and able to assure optimal fertility levels in cows. Its efficacy may be related to a quick absorption and diffusion of lecirelin through the CNS up to the pituitary gland.

References: (1) Grasselli *et al.*, 1996, *Zoot. Nutr. Anim.* **22**, 333.

Key Words: GnRH, LH, Progesterone, Cattle.

P105 THE EFFECT OF TWO DIFFERENT TEMPERATURE STEP DOWN PROGRAMS IN COMBINATION WITH DIETARY T_3 SUPPLEMENTATION ON GROWTH HORMONE AXIS IN BROILER CHICKENS. G. Rahimi*, M. Hassanzadeh** and E. Decuypere***, **Dept. of Ani. Sci., Univ. of Mazandaran, Iran; ** Facult of Vet. Med., Univ. of Tehran, Iran; ***Dept. of Ani., Sci., K.U.L, Belgium*

Body weight gain and its compositional changes are the result of complex interactions between endogenous and exogenous factors. Both genetic and environmental factors are translated into (neuro)endocrine signals which control and regulate intermediary nutrient metabolism. This experiment had factorial design with two levels of dietary T_3 (0 and

1ppm) which were administered from day 1 on and two different temperature step down programs (normal and low temperature) during first 3 weeks of post hatching age. Broiler chickens from a commercial line (Ross) were randomly divided into two rooms with two different temperature step down programs. In the normal temperature group the temperature decreased from 32°C on day one by 1°C every two days until a final temperature of 22°C was reached (day 21). In the low temperature group the temperature was lowered by 1°C every day down to final temperature of 22°C (day 11). Broiler chickens were fed a basal diet (3200 Kcal ME/Kg and 24% crude protein) with or without T_3. Each temperature-diet group consisted of 7 replicates (15 birds per replicate). Feed and tap water were available *ad libitum* and a nearly continuous lighting schedule (23L:1D) was provided for all birds. Birds and feed were weighed weekly and blood samples from individual birds were taken (10 birds per group) for assessing plasma growth hormone (GH) and insulin-like growth factor (IGF-I) content. Age-related changes in plasma GH were observed at all groups in the present experiment. Besides the obvious effect of age a clear difference was observed in body weight gain between the birds receiving control diet and those receiving a T_3 supplemented diet. The T_3 treated chickens had a significantly depressed growth rate. Feed intake and feed conversion ratio (FCR) increased as a function of age. No influence of ambient temperature on feed intake and FCR were found. T_3 treated diet significantly decreased feed intake but increased FCR during the growing period in normal and low temperature groups. The decreased growth rate or feed intake and increased FCR in broilers given T_3 supplemented diet confirmed earlier data (Decuypere *et al.*, 1994). Dietary T_3 decreased plasma GH levels in both temperature groups at all ages. No effect of ambient temperature was found on plasma GH levels. Plasma IGF-I levels were influenced by the T_3 supplemented diet. Broilers submitted to both normal and low temperature and fed T_3 treated diet showed lower plasma IGF-I levels compared with birds fed control feed. A decrease in plasma GH and IGF-I levels are in accordance with earlier studies (Wang *et al.*, 1989). The hyperthyroidism induced by dietary T_3 in combination with lower plasma GH and IGF-I levels may have contributed to a lower growth performance of broiler chickens in this study.

References: Decuypere *et al.*, 1994, *Brit. Poult. Sci.*, 35, 287; Wang *et al.*, 1989, *Poult. Sci., (Suppl. 1)*, 68, 154

Key words: Broiler, Dietary T_3, Growth performance, GH, IGF-I

P106 SERUM E2 AND LH LEVELS IN TRYPANOTOLERANT SOMBA COWS DURING SUPEROVULATIONS TREATMENT

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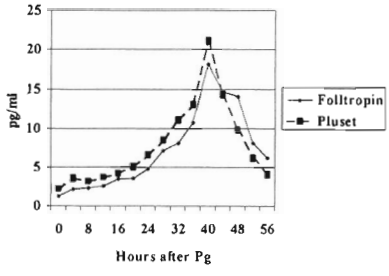
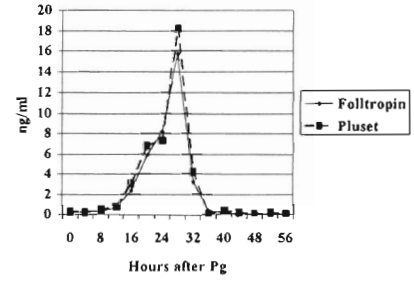
The aim of the work was to evaluate the reaction to a superovulation protocol of the Somba cow, an African cow bred in a restricted area of West Africa called "Somba country" between Benin and Togo.This breed is characterized by small dimensions (weight between 140 and 190 kg) and Trypanotolerance. Cows were treated with different p-FSH: two with Folltropin (Vetrepharm, Ireland) and two with Pluset (Serono, Italy). During superovulation (1) protocol blood samples were collected every 4 hours from Pg injection to the second artificial insemination. Serum was submitted RIA (2,3). Results are presented in figures 1 and 2.The low number of cows studied does not allow statistical analysis, but results obtained show that no difference in the treatment were noted.





References: 1) Cristofori F. et al., *Revue Elev. Méd. vét. Pays trop.* In press; 2) Bono G. et al., Theriog. 35 (6), 1179-1190 (1991); 3) Seren E. et al., Archivio Vet. Italiano 25,1-20.

Key words: E2, LH, trypanotolerant Somba, cows, superovulation

P107 QUANTIFICATION OF BST IN MILK BY ELECTROCHEMILUMINESCENCE: A WAY FOR DETECTION OF TREATED COWS. R. Renaville, D. Deaver*, C. Baronheid, C. Bertozzi, I. Parmentier, V. Haezebroeck, S. Fontaine, S. Hetzel & D. Portetelle. *Animal and microbial Biology Unit, Gembloux Agricultural University, Gembloux, Belgium, *PennState University, University Park, PA,USA.*

Somatotropine (ST) plays a key role in growth, lactation and metabolism in cattle. Since 1993, bST treatment is authorized in USA to promote milk synthesis in dairy cows while the European Community banned the hormonal administration at the same time. Also, the objectives of this research were a) to develop a sandwich immunoassay using a non-radioactive electrochemiluminescence (ECL) detection system, b) to compare ECL and ELISA methods and c) to determine if ECL method was able to identify bST treated cows using milk samples. 20 dairy cows were allowed in control (n=10) and biweekly bST treated cows (n=10). Hormonal treatment started at 70 d post-partum. Milk samples were collected on day 0, 14, 28, 63 and 133 after hormonal treatment. ELISA method for ST quantification was developed in our laboratory. Minimal detection was 500 pg/ml. Intra- and interassay coefficients were 5.8 and 9.8 %, respectively. In ECL method, a monoclonal anti-bovine ST antibody (6B1 clone) was labelled with N-hydroxysuccimide ester of a ruthenium tris-bipyridine chelate (RU). A second monoclonal anti-bovine ST antibody (8D5 clone) was labelled with biotine. Highly purified pituitary bovine ST (provided by the US National Hormone and Pituitary Program) served as standard. The labelled monoclonal antibodies and the standard (or sample) diluted with 0.05 M Tris-HCl buffer (pH = 7.5) containing 0.9% NaCl, 0.05% Triton X-100, were incubated overnight at room temperature. Antibody-antigen complexes were captured by addition of streptavidin coupled to magnetic beads (Dynabeads M-280, Dynal, Norway). ECL measurements were carried by the Origen-analyzer (Igen, USA). *Results.* Concentrations of ST were determined by ECL method in duplicated aliquots of 25 µl sonicated milk samples. Minimal detectability was 2 pg/ml in 200 µl assay volume corresponding to 10 pg/ml at 25 µl sample volume. Within-and between-assay coefficients of variation were 5.6 and 10.5%, respectively. Parallelism was assessed and verified between standard concentration and milk samples containing volumes ranging from 6.25 to 25 µl. Using this method, a significant difference ($P<0.05$ to $P<0.01$) appeared between control and bST treated cows. In untreated animals, milk ST concentrations fluctuated between 250 ± 30 and 330 ± 100 pg/ml during the experimental period while, after hormonal administration, ST concentrations in treated group were higher than 490 ± 150 pg/ml on average. In opposite, ST concentrations measured by ELISA method were at the detection limit and no difference were observed between the two experimental groups. This ECL-immunoassay provides a reliable and performant alternative to ELISA (or RIA), offers the major advantage of eliminating the use of radioisotopes, and is an interesting way to detect the bST treated cows using milk samples.

Key words: ECL, ELISA, bST, Milk, Cattle

P108 BETA-AGONIST ADMINISTRATION FOLLOWED BY DEXAMETHASONE TREATMENT DIFFERENTLY ALTERS SOMATOTROPIC AND THYROID AXIS. R. Renaville, I. Parmentier, V Haezebroeck, S. Fontaine, S. Hetzel, S. Massart, D. Portetelle. *Animal and microbial Biology Unit, Gembloux Agricultural University, B-5030 Gembloux, Belgium*

In a previous study, we have observed that clenbuterol decreased IGF-I synthesis while dexamethasone injection stimulated IGF-I production (Renaville *et al.*, 2000). The objectives of this study were to evaluate the effect of beta-agonist administration followed by corticoids injection, hormones frequently illegally used to promote growth performances, on the plasma insulin-like growth factor-I (IGF-I), IGF-binding protein-2 (IGFBP-2), IGFBP-3 and thyroid hormone (T_4, T_3) in finishing bulls. Twelve 350 days-old Belgian Blue bulls (mean weight: 331.2 ± 32.2 kg) were randomly assigned in 2 groups: a control group (C; n=6) and a group received *per os* a beta-agonist (clenbuterol, 5mg/day, CLEN) for d0 to d27 of the experiment followed by 3 *i.m.* injection of dexamethasone esters (Dexafort, 5ml, DEX) at d35, d42 and d49 (T; n=6). All animals received *ad libitum* a commercial diet. All animals were slaughtered on day 60. One blood sample was collected at weekly intervals. An additional 8-hr collecting blood period (one sample every 30 min) were realised on day 0, 14, 35, 50 and 57 of the trial. Circulating plasma IGF-I, IGFBP-2, IGFBP-3, T_3 and T_4 were measured by RIA methods. During the experimental period, the average daily weight gain statistically similar between C and T groups (1.43 +/- 0.33 kg/d vs. 1.55 +/- 0.41 kg/d, respectively). In T group, IGF-I plasma levels progressively decreased after CLEN administration and the difference with C group was significant at the end of the CLEN treatment period ($P<0.05$). After, DEX injection, quickly increased IGF-I levels to reach similar values than in C group. The plasma evolution in IGFBP-2 and -3 was different. Indeed, beta-agonist treatment stimulated IGFBP-2 and decreased IGFBP-3 synthesis while corticoids injection reduced IGFBP-2 and increased IGFBP-3 levels. The differences between C and T groups during CLEN treatment were significant ($P<0.05$) at day 14 and 27 while no difference appeared during the DEX period. If plasma T3 and T4 concentrations were unaffected by CLEN

administration, a significant reduction ($P<0.05$) was noted in T group after the third DEX injection. In conclusion, beta-agonist and corticoids differently altered IGF-I axis. Finally, these results also clearly confirm that plasma evolution of IGF-I and IGFBP-2 were opposite. (This work was supported by a grant from the Ministry of Small Enterprises, Traders and Agriculture (5736A)).

Reference: Renaville *et al.*, 2000, *Dom. Anim. Endocrinol* **18**, 165-176.

Key words: Bulls, Dexamethasone, Clenbuterol, IGF-I, IGFBPs, T_3, T_4

P109 RIA METHOD FOR PLASMA INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 QUANTIFICATION IN CATTLE AT VARIOUS PHYSIOLOGICAL SITUATIONS. R. Renaville, I. Parmentier, S. Hetzel, S. Fontaine, V. Haezebroeck, D. Portetelle. *Animal and Microbial Unit, Gembloux Agricultural University, 5030 Gembloux, Belgium.*

Insulin-like growth factor-I and –II (IGF-I, IGF-II) circulate in biological fluids bounds to at least six different IGF-binding proteins that regulate IGF bioactivity. Because bovine IGFBP-3 was unavailable from commercial societies, the objectives of the present study were to purified the protein from pre-colostrum, to develop an homologous radioimmunoassay and finally, to validate the method using plasma samples collected from animals in different physiological situations. The IGF-binding protein-3 has been purified by acid precipitation, molecular filtration and affinity chromatography from pre-colostrum milk collected 3-5 days before parturition. The affinity of the protein for IGF-I has been controlled as well as the purity of the preparation using Silver Stain method. The bovine IGFBP-3 preparation has been used to produce rabbit polyclonal antibodies and to develop a radioimmunoassay to quantify IGFBP-3 in bovine blood samples. Using the polyclonal antiserum, parallel displacement curves showed strong cross-reactivity with bovine, ovine, rabbit and human plasma protein and no cross-reactivity with porcine, rat, horse, dromedary or chicken plasma. Addition of IGF-I to a control pool of bovine plasma did not significantly alter control IGFBP-3 values in a radioimmunoassay. Minimal detectability was 1 ng/ml. Within and between assay coefficient of variation were 6.3 and 9.8 % respectively. During a nycthemeral period, plasma IGFBP-3 were stable and two or three samples were then sufficient to characterize the animal. During the onset of puberty in young bulls, IGFBP-3 increased in parallel with IGF-I and testosterone levels. Cows treated with recombinant bovine somatotropin (bST) had significantly higher plasma levels of IGFBP-3 than did control animals. Likewise, IGFBP-3 levels were dramatically decreased during the first postpartum weeks while propylene glycol administration during this period limit the fall of protein concentrations. This radioimmunoassay for bovine IGFBP-3, which enables quantitative assessment of IGFBP-3 concentration in cattle, confirmed the previous observations using the less precise Western ligand blotting method.
This research was supported by grants of the Belgian Ministry of Small Enterprises, Traders and Agriculture (# 5736A) and Walloon Region Ministry, subvention First Spin-Off # 991/3972.
Key words: Bovine, IGFBP-3, RIA, Plasma concentration

P110 EXPRESSION OF PROGESTERONE RECEPTORS AND mRNA IN BOVINE *CORPUS LUTEUM* DURING ESTROUS CYCLE. R. Tamane[1], M. Pilmane[2], A. Jemeljanovs[1] [1]*University of Agriculture, Research Center "Sigra", Instituta Street 1, Sigulda, LV 2150,* [2] *University of Latvia, Department of Anatomy and Histology, Raina bulv. 19, Riga, LV 1586, LATVIA*

The *corpus luteum* (CL) is a functional structure in ovaries for producing progesterone (PR), which regulates normal function of ovaries in estrus. The proposed auto regulatory role of progesterone action in the CL is bound with the expression and function of intracellular progesterone receptors (Ottander et al., 2000). We investigated the expression of progesterone receptors (PR Rec) and mRNA in 23 cyclic bovine *corpora lutea*. 4 of all were developing *corpora lutea* (1. -- 7. day of estruos cycle), 8 were mature *corpora lutea* (8. – 16. day of estrous cycle), 7 were *corpora lutea* in early regressing stage (16. – 21. day of estrous cycle), 4 were regressed *corpora lutea*. CL were obtained from slaughter animals, fixed in solution Stefanini and embedded into paraffin. PR Rec expression was detected by use of biotin streptavidin immunohistochemistry. mRNA expression was examined by Unna-Brashe method. The highest expression of PR Rec was observed in cells of matured CL (5.4 ± 1.2 positive cells in visual field). Decreased expression of progesterone receptors was detected in luteal cells of developing CL, but significant decrease showed cells of early regressing stage (2.8 ± 0.4 positive cells in visual field) and regressed CL (0.7 ± 0.4 positive cells in visual field), ($p < 0.05$). The highest expression of mRNA was observed in cells of developing CL (11.5 ± 0.4 positive cells in visual field). The expression of mRNA in cells of mature CL declined significantly ($p < 0.05$). Occasional cells showing very low level of mRNA activity we observed in cells of regressed CL (1.7 ± 1.7 positive cells in visual field). It was

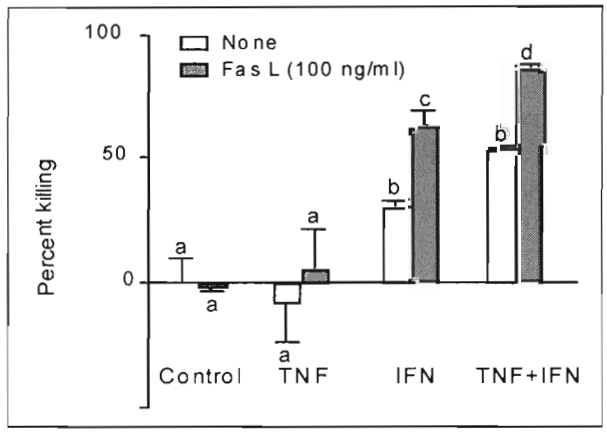
animals, fixed in solution Stefanini and embedded into paraffin. PR Rec expression was detected by use of biotin streptavidin immunohistochemistry. mRNA expression was examined by Unna-Brashe method. The highest expression of PR Rec was observed in cells of matured CL (5.4 ± 1.2 positive cells in visual field). Decreased expression of progesterone receptors was detected in luteal cells of developing CL, but significant decrease showed cells of early regressing stage (2.8 ± 0.4 positive cells in visual field) and regressed CL (0.7 ± 0.4 positive cells in visual field), ($p < 0.05$). The highest expression of mRNA was observed in cells of developing CL (11.5 ± 0.4 positive cells in visual field). The expression of mRNA in cells of mature CL declined significantly ($p < 0.05$). Occasional cells showing very low level of mRNA activity we observed in cells of regressed CL (1.7 ± 1.7 positive cells in visual field). It was significantly lower than that in cells of early regressing stage CL ($p < 0.05$). A strong correlation between both parameters in estrous cycle was found ($r = 0,8$). In conclusion, our data suggest that like in human CL (Duffy et al. 1995, Hid-Petito et al. 1997), the highest expression of PR Rec is observed in the cells of mature CL, but numerous cells with morphofunctional activity were found in developing CL. These findings suggest an asynchrone regulation of PR and mRNA in bovine CL during estrous cycle.

References: Duffy et al., 1995, Endocrinol. 136, 1869; Hid-Petito et al., 1997, J. Clin. Endocrinol. Metab. 82, 955; Ottander et al., 2000, Biol. Reprod. 62, 655

Key Words: progesterone receptors, mRNA, corpus luteum

P111 **FAS/FAS LIGAND SYSTEM MEDIATES STRUCTURAL LUTEOLYSIS IN BOVINE CORPUS LUTEUM.** H. Taniguchi, K. Okuda. *Laboratory of Reproductive Endocrinology, Faculty of Agriculture, Okayama University, Okayama 700-8530, Japan.*

Structural regression of corpus luteum (CL) occurs by apoptosis. Fas antigen (Fas) is a cell surface receptor that triggers apoptosis in sensitive cells when bound to the Fas ligand (Fas L). The purpose of the present study was to identify the presence of a Fas/Fas L system in bovine CL and to evaluate the regulation of Fas-mediated luteal cell death by immune cell-derived cytokines. Bovine luteal cells from mid-cycle CL (Days 8-12 of the estrous cycle) were exposed for 24 h to interferon- . IFN; 50 ng/ml) and/or tumor necrosis factor- . (TNF; 50 ng/ml). After 24 h of culture, the expression of Fas mRNA was detected in cultured bovine luteal cells and was increased by IFN. Moreover, TNF augmented the stimulatory action of IFN, whereas TNF alone did not affect the expression of Fas mRNA. The effects of IFN and TNF on Fas-mediated cell death were also examined. Cells were exposed to IFN and/or TNF for 24 h, and were then treated with IFN and/or TNF in the presence or absence of Fas ligand (100 ng/ml) for 24 h. Treatments of the cells with IFN alone and in combination with TNF resulted in killed 30% and 50% of the cells ($P<0.05$), respectively, whereas TNF alone did not have a cytotoxic effect on the cells. On the other hand, Fas L killed 60% of the cells treated with IFN ($P<0.01$) and 85% of the cells treated with the combination of TNF and IFN ($P<0.01$), respectively, whereas Fas L had no effect on the viability of the luteal cells treated with or without TNF. Furthermore, shrunken nuclei and apoptotic bodies were observed in the cells treated with Fas L in the presence of TNF and IFN. These results suggest that a Fas/Fas L system is present in bovine CL, and that immune cell-derived cytokines play important roles in Fas-mediated luteal cell death.



Key Words: Cattle, Corpus luteum, Apoptosis, Fas/Fas ligand system

P112 DEXAMETHASONE REDUCES ACROSIN ACTIVITY OF RAM SPERMATOZOA M.P. Tsantarliotou[1], I.A. Taitzoglou[2], N.A .Kokolis[1] [1] *Department of Physiology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 540 06 Thessaloniki, GREECE;* [2] *Department of Physiology, Faculty of Veterinary Medicine, University of Thessaly, 431 00 Karditsa, GREECE*

Acrosin is a sperm acrosomal serine proteinase involved in the early stages of fertilization. Acrosin activity in ram spermatozoa reveals a seasonal variation in various breeds; testosterone parameters also exhibit seasonal variation and are in a positive correlation with acrosin activity of spermatozoa. Glucocorticoids released during stress influence blood testosterone, directly or indirectly. The aim of the present study was to investigate the possible effect of dexamethasone (DEX), a synthetic glucocorticoid, on acrosin activity of ram spermatozoa during autumn (breeding season for sheep in Greece) in correlation with possible changes in blood testosterone. For the purpose of this study 8 one year old Chios rams were used. The animals were divided into 2 groups, treated (n=4) and controls (n=4). DEX was administered in four equal consecutive intra-muscular injections, one every four hours (total dose: 3mg/kg). Semen samples, from each ram, were collected for acrosin activity assay, 48 hours before the administration of DEX, and on days 4, 7 and thereafter once weekly until the 77th day after administration. Acrosin activity of spermatozoa was determined spectrophotometrically. In parallel, blood samples for testosterone assays were collected 24 hours before administration (one sample/30min x 7.5 hours), during administration (one sample/30min x 32 hours), and on days 4, 7, 14 and 21 after administration. Plasma testosterone concentration was determined by RIA. For each ram, six parameters of testosterone were estimated: mean value, basal level, number of peaks, peak amplitude, peak duration and mean testosterone concentration during peaks. Total acrosin activity was reduced between days 7-28 after DEX administration. DEX also induced a reduction of mean value and basal level of blood testosterone and inhibited its episodic secretion 1-4 days after administration. No significant correlations were observed between total acrosin activity and testosterone parameters in control and DEX treated rams. As the reduction of acrosin activity appeared relatively soon after DEX administration (7th day), it is likely that the increased amount of DEX did not influence the synthesis of proacrosin/acrosin in the late spermatids. As glucocorticoid receptors exist in the epididymis and accessory glands in various species, DEX might influence the synthesis and/or release of acrosin inhibitors in epididymal fluid or seminal plasma. These changes of acrosin activity in the ejaculated ovine spermatozoa induced by DEX might be of importance regarding the role of stress in the reduction of sperm fertilizing ability.

Key words: glucocorticoids, semen, testosterone, proteolytic enzymes.

P113 EFFECT OF REFEEDING ON GROWTH HORMONE EXTRA- (GHRe) AND INTRACELLULAR DOMAIN (GHRi) GENE EXPRESSION IN CHICKEN LIVER. P Van As, V Beck, O.M. Onagbesan, V Bruggeman, S. Van Der Geyten, V Darras, E. Decuypere. *Poultry Research Group, Faculty of Agricultural and Applied Biological Sciences, Katholieke Universiteit Leuven, Kasteelpark Arenberg 30, B-3001 Leuven, Belgium.*

The GHR consists of extra- and intracellular parts and belongs to the cytokine receptor superfamily, a family of single membrane-spanning receptors, which shares considerable amino acid sequence homology of conserved regions. The family also includes prolactin receptors, erythropoietin receptors and several other cytokines receptors (Hochberg *et al.*, 1991). The existence of soluble, truncated forms of receptor, referred to as binding proteins, is a common feature of many members of this superfamily. The expression of GHR is regulated by different factors, such as GH concentration, steroid hormones, and hormonal and/or metabolite factors related with food uptake. Current methods for quantifying GHR gene expression, including mRNA blots, image analysis of in situ hybridisation are limited in terms of sensitivity. Moreover, many of these studies concentrated on quantifying either the soluble (GHBP) or the whole GHR but not both extracellular (GHRe) or intracellular domains (GHRi). For this purpose, we have used the more sensitive competitive reverse transcription-polymerase chain reactions (RT-PCR) specific for the intracellular and the extracellular part of the chicken GHR to quantify both domains in the liver of chickens (Van As *et al.*, 2001). Expression levels of GHR were determined from standard curves generated and food deprivation and refeeding were used as experimental model. An experiment with chickens that received a different food schedule after two weeks, was set up. One group was fed ad libitum (AL), the other was food restricted (FR) (they were fed once a day during half an hour). After 4 weeks the chickens were slaughtered and the liver was removed. Several samples were taken at different time points just before (0 minutes) and after feeding (60, 90, 120 and 200 minutes). The total RNA was isolated out of the liver. When plotting the GHRe mRNA concentration of FR and AL chickens as a function of time, the initial concentration of FR chickens was lower compared with the AL chickens. The GHRe mRNA concentration of all FR chickens then increased after feeding as a function of time but never reached the level of the AL chickens. After this increase in some cases already a decline was observed at 200 minutes in some animals but not in others. Food deprivation increased plasma GH concentrations in growing broiler chickens, but decreased GH-dependent variables such as plasma insulin-like growth

factor I and 3,3',5-triiodothyronine concentrations (Buyse *et al.*, 2000). This can be a consequence of the low hepatic GHR numbers we could detect concomitant with low GH gene expression. Food intake reversed these variables in a time-related manner.

References: Buyse *et al.*, 2000, Britisch Poultry Science **41**, 107; Hochberg *et al.*, 1991, *Cell. Signalling* **3**, 85; Van As *et al.*, 2001, *Gen. Comp. Endocrinol.* **122**, 213.

Key Words: GHR, Competitive RT-PCR, Refeeding

P114 THE EFFECT OF THE WEIGHT AND AGE OF GILTS AT THE FIRST FERTILIZATION AND THE AGE OF SOWS AT THEIR FIRST FARROWING ON THE NUMBER OF PIGLETS AND LITTER WEIGHT AT BIRTH AND WEANING.

N.Varatanovic, M.Podzo**, E.Karahmet***. *Veterinary faculty Sarajevo.*

Depending on the proportion of gilts in the structure of a herd, in sows farrowed for the first time all the reproductive parameters decrease to a larger or smaller extent, thus causing the production results at the farm. Therefore the knowledge of individual factors influencing the fertility in the first and in later bearings with piglets is extremely important. During the first fertilization process, gilts age and, in some authors opinion, their weight as well, have very significant impacts on reproduction parameters. Miskovic states that the age of gilts is a more important criterion than the body weight in determining gilts reproductive maturity. From the point of view of economy, some authors find that the best measure of reproductive efficacy would be the number of feeding days or the productive age of a sow per a live-born piglet. Milena Kovac and Salehar state that the number of feeding days of sows per a live-born piglet at Slovenian farms is between 14.68 and 22.33 days. The investigation on the influence that age and weight have on the number of piglets and the litter weight at birth and weaning has been conducted on approximately 2000 gilts at a farm. After ultrashalling, determining the selection index and health evaluation, gilts have been led into reproduction and divided in groups of 30 to 35 heads each. The age of selected gilts was between 185 and 235 days and their weight was between 95 and 120 kilograms. During the research the weight and age of gilts (first-time-fertilized sows), number of live and still -born piglets, as well as the litter weight on the first and twenty-eighth day after farrowing were registered. All the collected data were analysed using the Last Squares Method -SSQ, Harvey 1981. There are different opinions about the maturity and physical development of gilts which are to be led into reproduction. Gilts are mostly led into reproduction at the body weight between 90 and 110 kilograms, as stated by Miskovic et al. When comparing results related to the age of the first-time-fertilized gilts, one can recognize differences between various groups, what is in agreement with the findings of majority of authors. According to our results, the largest number of live-born piglets – 8.38 was given by gilts fertilized at age of 196 to 220 days, while the age of gilts did not have any influence on the number of still-born piglets and the litter weight at birth had the same tendency as live-born piglets. The existence of a significant difference of litter weight in the period of weaning at age of 196 to 220 days we tend to ascribe to a larger number of weaned piglets. Exploring this problem further on, many authors have come up with the data about the age of the first-time-fertilized gilts and their productive capability which are somewhat different from ours.

References: Kovac Milena and Salehar A., Beograd 1986. Proizvodnja svinja u mediteranskim zemljama, Miskovic et al. Skopje 1977., V Sobir za odgeduvacite na svinji na Jugoslavija.

Key Words: gilt, weight, age, piglets number, litter weight

P115 DIFFERENTIAL EFFECT OF INTRAVENTRICULAR INFUSIONS OF NEUROPEPTIDE Y ON LH AND FSH PITUITARY SECRETING CELLS IN PREPUBERTAL FEMALE LAMBS.

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Neuropeptide Y (NPY) is a putative neuroregulator of the reproductive axis on the level of the central nervous system. The present study was designed to demonstrate whether exogenous NPY infused to the 3rd ventricle of the brain can affect the secretory activity of gonadotropic cells in the pituitary gland of prepubertal lambs. Immature female Merino sheep (n=12) at the age of 34 weeks, before their first estrous cycle were subjected to the implantation procedure. Next, Ringer-Locke solution (control) or 50 μg of NPY were infused for 5 min. to the 3rd ventricle of the brain and sheep were slaughtered 3 h after infusions. Immunoreactive (ir) LH- and FSH- producing cells were localized by immunohistochemistry using antibody raised against LHβ and FSHβ. Messenger RNAs analyses were performed by nonisotope hybridization *in situ* technique using sense and antisense riboprobes produced from β subunits of LH and FSH cDNAs clones. The results were generated by computer image analysis for the percent of immunoreactive and/or

hybridizing cells and optical density for immunostaining and hybridization signal. LH in the blood plasma was determined by radioimmunoassy. It was found that in the lambs infused with NPY, a number of immunoreactive and hybridizing LH cells increased significantly ($P<0.001$) by 64% and 52% respectively, compared to the vehicle infused animals. Furthermore, the prominent increase ($P<0.001$) of optical density for immunostaining and hybridization signal were observed in both, the irLH cells and mRNA LHβ-expressing cells after NPY infusions by 55% and 124% respectively. The concentrations of LH in the blood plasma did not differ between treatment groups (2.66 ± 0.4 vs 2.78 ± 0.3 ng/ml). The NPY infusions displayed no effects on the immunoreactivity of FSH cells or on expression of mRNA for FSHβ. It is concluded that NPY may be an important component of mechanisms stimulating the LH synthesis and storage but not the release in the pituitary cells in prepubertal female sheep. In addition, this effect is specific to LH, no such effect was observed on FSH secretion.

Key Words: NPY, gonadotropic cells, puberty, ovine.

P116 PRE-CHALLENGE ADMINISTRATION OF GRF REDUCES VARIATION IN GROWTH HORMONE RESPONSE TO GRF IN GROWING HOLSTEIN HEIFERS. W. J. Weber, S. Cieslak, and B. A. Crooker. *Department of Animal Science, University of Minnesota, St. Paul. USA.*

Growth hormone (GH) response to administration of growth hormone releasing factor (GRF) has been used in attempts to distinguish between genetically inferior and superior animals. However, the relationship between GH response and genetic merit has not been consistent. This inconsistency is due, in part, to the large within animal variation associated with this technique. The objectives of this study were to determine the variation in GH response to GRF within an animal on successive days and if a pre-challenge flushing dose reduced this variation. Catheters were implanted in both jugular veins of six month old Holstein heifers (N=8) on day 0. Each heifer received 4 ug/100 kg BW of human GRF (1-29) analog (Hoffman-LaRoche, Ro23-7863) at 0 and 120 min of the sampling period on day 1, 2, and 3. Blood samples were obtained at -30, -20, -10, -5, 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 90, 100, 110, 115, 120, 122.5, 125, 127.5, 130, 135, 140, 150, 165, 180, and 210 min. Concentrations of GH were determined by RIA. Mean pre-challenge GH concentration (PCGH) was determined during the 30 min interval prior to each GRF administration. Area under the GH response curve was quantified (0 to 45 min post-dosing, AUC45) by trapezoidal summation between successive pairs of GH concentrations and time coordinates after subtracting PCGH. Effect of day, challenge and their interaction was assessed using GLM of SAS. Means differed when $P < 0.05$. Pre-challenge GH did not differ between challenges (5.2 and 6.2 0.58 ng/ml) or among day of challenge (5.2, 5.7, 6.4 0.72 ng/ml). Response to the first challenge was greater than response to the second challenge (1381^a, 909^b 139 ngminml^{-1}). On day 1, AUC45 was less than on day 2 or 3 (773^a, 1373^b, 1288^b 171 ngminml^{-1}). Mean AUC45 for the first challenge (762 170, 1806 253, 1575 363 ngminml^{-1}) varied more among days than the second challenge (784 203, 941 116, 1002 265 ngminml^{-1}). On day 2 and 3, variation within day was greater for the first than the second challenge. Results indicate GH concentrations were similar to PCGH concentrations within 90 min after the first GRF challenge and that the flushing dose reduced variation in GH response. The ability of this reduced variation to enhance detection of treatment differences needs to be evaluated.

Key Words: GRF challenge, GH, Holstein

P117 EFFECT OF INTRAVENTRICULAR INFUSIONS OF NEUROPEPTIDE Y ON GnRH/LH SECRETION IN THE EARLY ANESTROUS PERIOD OF EWES. A.Wójcik-Gładysz, T. Misztal, M. Wańkowska, J. Polkowska. *The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland*

Neuropeptide Y (NPY), being widely distributed in the mammal's brain is a putative mediator of many physiological processes including reproductive functions. The aim of present study was to estimate the role of exogenous NPY infused to the 3rd ventricle of the brain of sheep in the early anestrus, i.e. the period of diminishing reproductive activity, on the hypothalamic GnRH neurons, pituitary LH producing cells and plasma LH levels. The experiment was performed on crossbreed sheep (n=14) subjected to the implantation procedure to the 3rd ventricle. The five min. infusions of Ringer-Lock's solution (RL, controls) or NPY (50ug/200ug RL) were carried out in 1st and 6th week after the last estrous cycle in the four groups of animals. On the day of infusion blood plasma was collected every 10 min. for 3 h before and after infusion and then ewes were slaughtered and brains were fixed *in situ*. GnRH in the hypothalamus and LH in the pituitary gland were estimated by immunohistochemistry (ICH) and image analysis, while LH in the blood plasma by radioimmunology. ICH analysis showed that in the sheep being in both, 1st and 6th week of inserts,

immunoreactive (IR) GnRH was present in the median eminence (ME), however, their content was significantly lower (P<0.001) in sheep in 6th than in 1st week of anestrus. The number of IR LH cells in pituitaries of sheep in both anestrous phases did not differ but the content of IR LH was significantly lower in the latter phase (P<001). LH concentrations and pulse frequency were similar in both RL infused groups (4.5 ng/ml and 2 pulses/3 h). NPY infusions induced significant (P<0.001) decrease of IR content of GnRH in the ME (by 72%), IR content of LH in the pituitary cells (by 47%), the number of IR LH cells (by 34%) and increased (P<0.05) plasma LH concentrations within 3 h after infusions (4.52 ± 1.05 vs 5.13 ± 1.1 ng/ml) in the sheep in the 1st week of anestrus. No changes were observed after NPY infusions in the IR GnRH and IR LH content, in the number of IR LH cells, as well as in LH concentrations in the sheep in 6th weeks of anestrus. The presented results imply, that NPY can play a modulatory function in the regulation of the GnRH/LH secretion in the sheep on the central nervous system level. The sensitivity of this secretory system to this presumably NPY modulation seems to be dependent on reproductive activity in this species.
Key Words: GnRH/LH, NPY, anestrus, ewe.

P118 COMPARISON OF SERUM SOMATOTROPIN, THYROXINE, TRI-IODOTHYRONINE, FREE FATTY ACIDS, TOTAL PROTEIN LEVELS AND LIVE WEIGHTS IN NEW-BORN SAKIZ LAMBS SUCKLING MOTHER AND FEEDING ARTIFICIALLY. A.Fırat, A. Özpınar, A. Ateş, B. Serpek*, S. Haliloğlu*. *University of Istanbul, Istanbul, Turkey and * University of Selçuk, Konya, Turkey.*

Growth is a complex process controlled by a hormonal system termed somatotropic axis. Somatotropin is a protein hormone secreted by acidophyllic cells in anterior lobe of hypophysis. It is supposed that a correlation may be between growth performance and plasma somatotropin levels (Scanes et al., 1987). The aim of this study was to determine the effects of different feeding and age on serum somatotropin levels in twin Sakız lambs. In the study, 20 new-born Sakız lambs were used. Lambs born from the same mother were equally divided into two groups. 10 lambs in group 1 were immediately separated from their mothers after parturition and fed commercial cow milk and milk replacer. 10 lambs in group 2 were suckled their mothers. Blood samples were taken from lambs 12 hr, 24 hr, 48 hr, 14 d, 28 d, 42 d, 56 d, 90 d and 120 d after parturition. Serum somatotropin levels were analysed by enzymeimmunoassay method. Serum thyroxine and tri-iodothyronine levels were analysed by RIA. Serum free fatty acid and total protein levels were analysed spectrophotometrically. Live weight, serum somatotropin, free fatty acids, total protein and tri-iodothyronine levels tended to be higher in lambs suckled their own mothers than separated lambs. Plasma growth hormone concentrations are very high in fetus, suddenly fall during perinatal period and after a transient increase, decrease with advancing age (Claus and Weiler, 1994). Rhind et al. (1992) reported that following the daily introduction of fresh food, there was a decrease in mean concentration of somatotropin. In the study, changeable somatotropin levels were determined, the lambs were in growth period and somatotropin levels were high in growing lambs.
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Key Words: Somatotropin, thyroid hormones, free fatty acids, lamb.

P119 ANGIOGENESIS IN THREE-DIMENSIONAL FIBRIN GELS: CO-CULTURE OF PIG GRANULOSA CELLS WITH ENDOTHELIAL AORTIC CELLS IN A MICROCARRIER-BASED SYSTEM. Tirelli M., Cavalli V., Grasselli F., Bussolati S., Basini G., Tamanini C.. *Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualita' e Sicurezza degli Alimenti, Sezione di Fisiologia Veterinaria, Universita' di Parma, 43100 Parma, Italy.*

Ovarian follicles can control the growth of their own capillary beds by producing angiogenic factors such as Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF). We have recently reported (Basini et al., 2000) that granulosa cells collected from antral follicles represent a source of VEGF, whose levels are positively related to follicle size The aim of the present work was to develop a reliable in vitro system in order to determine the angiogenic activity of granulosa cells; to do this, granulosa cells were co-cultured with aortic endothelial cells (PAEC) in a microcarrier-based in vitro assay which allows the study of capillary growth in three dimensions. Briefly, swine granulosa cells collected from small (<3 mm), medium (3-5 mm) or large (>5 mm) follicles were seeded in pre-treated collagen wells at a density of 10^6 cells/well and cultured for 48 h in M199 with BSA 0.1% and FCS 1%. For endothelial cell culture, passage 10 trypsinized cells, obtained from porcine aortas, were allowed to attach onto cytodex-3 microcarrier beads for 4 h and subsequently were grown for 24 h at 37°C; for co-culture, 40 µl of the microcarriers coated with PAEC were added to granulosa cell wells after culture media were discarded, and embedded in three-

dimensional fibrin gels as described by Nehls and Drenckhahn (1995), with some modifications. Co-cultures of granulosa cells and PAEC were incubated at 37 °C in 5% CO_2 for 4 days; the culture media were renewed every other day. Using the same fibrin gels, control cultures of PAEC were incubated with different concentrations of VEGF or Endothelial Cell Growth Supplement (ECGS). The angiogenic activity of granulosa cells was determined by evaluating the number as well as the length of the capillary like structures derived from the cell-coated microcarriers after 24, 48, 72 and 96 h of co-culture. Granulosa cell angiogenic activity appeared to vary according to follicle size: while endothelial cells co-cultured with cells from small and medium follicles significantly migrated into the fibrin matrix and formed sprouts, co-cultures with cells from large follicles inhibited capillary formation and elongation. Data from this and previous (Basini et al., 2000) experiments suggest that ovarian angiogenesis is a multifactorial process whose mechanisms are far to be elucidated. *This work was supported by a MURST COFIN grant.*

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Key words: pig, angiogenesis, endothelial cells, granulosa cells.

P120 EFFECT OF A SINGLE DOSE OF IBUPROFEN LYSINATE BEFORE EMBRYO TRANSFER ON PREGNANCY RATES IN COWS. M. Elli , B Gaffuri, A Frigerio, M. Zanardelli, D. Covini, M. Candiani and M. Vignali . *II Department of Obstetrics and Gynecology,University of Milano , via commenda 12, 20122 Milano, Italy; Istituto Auxologico Italiano, Milano, Italy; Division of Animal Production,Como, Italy;and LisaPharma, Erba,Como, Italy.*

Embryo implantation is a critical step in both cows and humans. The use of ibuprofen lysinate to enhance implantation has been investigated in cattle with the specific aim of improving pregnancy rates after embryo transfer.
In this study, heifers (n 100) were assigned randomly to one group: one group was treated i.m. with 5 mg ibuprofen lysinate Kg body weight 1 h before embryo transfer and a control group received vehicle only.A single embryo was transferred into each recipient cow. There was a significant difference in the number of pregnancies after embryo transfer between cows in the treated (41 of 50; 82%) and control (28 of 50; 56%)groups. These data indicate that ibuprofen lysinate may be an effective adjunctive treatment for assisted reproducyion in cattle . Further studies are needed to clarify whether this effect is associated with with the reduction of cyclooxygenase enzyme isoforms during embryo transfer or whether other mechanisms are involved.

Keywords: hybuprofenlysinate, embryotransfer, pregnancy