1: Almášiová, V.[1] – Cigánková, V.[1] – Holovská, K. jr.[1] – Škrobánek P.[2] – Račeková E.[3]:

INFLUENCE OF HYPODYNAMY ON SPERMATOGENESIS AND STEROIDOGENESIS OF JAPANESE QUAILS

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[2]Institute of Animal Biochemistry and Genetics, Ivanka pri Dunaji, Slovak Republic;
[3]Institute of Neurobiology SAV, Košice, Slovak Republic]

This investigation was carried out to observe the effect of simulated weightlessness on spermatogenesis and steroidogenesis of Japanese quails. Two days after hatching, the quails were placed into special individual suspender scaffolds, so their feet did not touch the floor. They could consume food and water *ad libitum*. Experimental animals were sacrificed after 14, 21, 28, 35, 42, 49 and 56 days of hypodynamy. Samples of testes were processed for light (LM) and transmission electron microscopy (TEM). The structure and ultrastructure of the testes in experimental animals were disturbed. Spermatogenesis was retarded in experimental animals compare to their control.

2: Fazekašová, J.[1] – Makarevič, A. V.[2] – Sirotkin, A. V.[2] – Bulla, J.[1] – Pivko, J.[1,2]:

THE EFFECT OF GHRELIN ON FUNCTIONS OF BOVINE OVIDUCTAL CELLS *IN VITRO*

[[1]Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovak Republic;
[2]Animal Production Research Centre Nitra, Slovak Republic]

The aim of our in vitro experiments was to investigate the role of ghrelin (Ghr), a newly discovered metabolic hormone in control of proliferation (PCNA, cyclin B1), apoptosis (Bax, p53) and secretory activity (IGF-I and PGF 2α) of bovine oviductal cells *in vitro*. The cells were isolated by flushing oviducts from slaughtered cows. The cells were pre-cultured in DME/F12 medium containing 20% FCS and 1% antibiotic-antimycotic solution. After 2 days, the medium was changed for fresh medium containing 20% FCS and Ghr at doses 0, 1, 10 or 100 ng.ml^{-1}, and the cells were cultured for 72 hours. Concentrations of insulin-like growth factor (IGF-I) and prostaglandin F2α (PGF2α) in cell-conditioned medium were measured by IRMA/RIA kits. Indices of cell apoptosis (apoptotic peptides Bax and p53) or proliferation (the proliferation associated peptide PCNA and cyclin B1) in the bovine oviduct epithelial cells were analyzed by immunocytochemistry (ICC) and Western-blotting (WB). It was observed that Ghr stimulated PGF 2α (at all tested doses; $p<0.05$) and IGF-I output (at 1 and 100 ng.ml^{-1}; $p<0.05$) by the oviductal cells. Ghr, given at all doses, increased the proportion of PCNA-positive cells (ICC, WB). The proportion of Bax-, cyclin B1- and p53- positive cells (ICC) was not changed significantly after addition of Ghr. These results clearly demonstrate that Ghr is a potent regulator of the secretory activity and proliferation in oviduct epithelial cells. Elucidation of the role of Ghr in apoptosis regulation within the oviduct must await further investigations.

Supported by the Grant Agency for Science of Slovak republic VEGA, Slovak Republic (grant no. 1/0834/08)

3: Franczak, A. – Kurowicka, B. – Żmijewska, A. – Wojciechowicz, B. – Kotwica, G.:

EFFECT OF OXYTOCIN (OT) ON PGE2 SYNTHESIS AND SECRETION BY PORCINE MYOMETRIUM DURING EARLY PREGNANCY AND THE ESTROUS CYCLE

[University of Warmia and Mazury in Olsztyn, Poland]

Past studies of uterine prostaglandin (PGs) in pig reproduction documented that porcine myometrium preferentially produces PGs during early pregnancy and secretes more luteotrophic PGE2 than luteolytic PGF2α. This study documents the involvement of oxytocin (OT) in myometrial synthesis and secretion of PGE2. Post-pubertal crossbred pigs (Large White x Polish Landrace) weighing 90-110 kg were used during the estrous cycle or early pregnancy. The animals were slaughtered on days 10–11 (n = 5), 12–13 (n = 5) and 15-16 of pregnancy (n = 5) and the estrous cycle. The myometrium was minced into small slices (200-210 mg, 3 mm thick) and pre-incubated in medium M199 for 18 h (37 ºC, 95% O_2 and 5% CO_2). These preincubated slices were treated for 6 h and 12 h with control medium and OT (10-7M). After incubation, culture vials were placed in an ice bath and medium was collected and frozen at -20 ºC until EIA PGE2 assay. Expression of the mRNA encoding microsomal PGES-1 (mPGES-1) in porcine myometrium during early pregnancy and the estrous cycle was investigated by a quantitative Real-Time PCR. A Quantitative Real-Time PCR was performed using SYBR Green Mix × 1 (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Primers for mPGES-1 forward-5' CCAAGTGAGGCTTCGGAAGAAG and mPGES-1 reverse-5'CCAGGTAGGCTATGGTGTG 3' amplified a 252 -base pair (bp) fragment of the mPGES-1 gene (AY863054495). Primers for the housekeeping gene glyceraldehydes-3-phosphate dehydrogenase (GAPDH) forward -5' CCTTCATTGACCTCCACTACATGGT 3' and GAPDH revers – 5' CCACAACATACGTAGCACCACGAT 3' amplified a 183 -base pair (bp) fragment of the GAPDH gene (U48832). Results: 1) OT did not affect abundance of mRNA for mPGES-1 during early pregnancy; 2) In response to OT, myometrial abundance of mRNA for mPGE-1 on days 15-16 of the estrus cycle was decreased; 3) Myometrium produced higher amounts of PGE2 on days 12-13 of pregnancy compared with respective days of the estrous cycle; 4) OT increased secretion of PGE2 from myometrium during studied days of early pregnancy and on days 12-13 of the estrous cycle. In conclusion: oxytocin acts on the porcine myometrium as a stimulator of luteotrophic PGE2 secretion from this tissue during early pregnancy.

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4: Jabłońska, O. – Piasecka, J. – Ciereszko, R. E.:

THE ARYL HYDROCARBON RECEPTOR (AhR) mRNA EXPRESSION IN THE REPRODUCTIVE TRACT OF THE PIG

[Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland]

The aryl hydrocarbon receptor (AhR) has been

recognized as a mediator of xenobiotic-induced toxicity. In addition, it was demonstrated that the AhR may play a significant role in the regulation of reproductive processes in females. The aim of the study was to examine the expression of AhR mRNA in different parts of the porcine reproductive tract (follicle, corpus luteum, ovarian stroma, oviduct, endometrium, myometrium) during the follicular (Days 16-19) and luteal (Days 10-12) phase of the estrous cycle. The examined tissues originated from pigs with two controlled cycles; following dissection, the tissues were immediately frozen in liquid nitrogen and stored at -80 ºC. After extraction of total RNA, the first strand cDNA was generated from 1 µg of RNA. TaqMan gene expression assays containing primers and probes for porcine AhR and β-actin were used in quantitative real-time PCR. The relative gene expression was calculated using ΔΔCt method. Each sample was run in duplicate. One-way ANOVA followed by the least significant difference (LSD) post hoc test was used to analyze the expression of AhR mRNA in porcine reproductive tissues. Differences with a probability of $p<0.05$ were considered significant. Examination of tissues collected during the follicular phase (n=4-6) revealed that the lowest level of AhR mRNA was found in ovarian follicle and myometrium, while the highest levels were demonstrated in the oviduct, endometrium and ovarian stroma ($p<0.05$). The expression of AhR mRNA during the luteal phase (n=4-6) was the lowest in the uterus. The highest levels were observed in the corpus luteum and ovarian stroma. The AhR mRNA expression was 8-fold higher ($p<0.05$) in the corpus luteum in comparison with the uterus. The results demonstrate that AhR transcript is expressed in the reproductive tract of the pig. It also suggests that AhR may play a significant role in the regulation of female reproduction.

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5: Júdová, J.[2] – Šak, D.[1]:

THE INFLUENCE OF LEAD ON CRUSTACEANS IN HIGH MOUNTAIN ENVIRONMENT

[[1]The Institute of High Mountain Biology of University of Žilina, Slovak Republik, Tatranská Javorina; [2]Matej Bel University, Faculty of Nature Sciences, Banska Bystrica, Slovak Republic]

The main aim of this work is to monitor the influence of lead on the selected species of crustaceans, Cyclops abyssorum tatricus. Through genetic methods such as DNA isolation, polymerase chain reaction, and agarose gel electrophoresis, changes in individual gene selection were monitored dependant on the concentration of lead acetate. The number of days individuals survived was lower with remaining concentrations of lead acetate. A higher concentration of lead acetate decreased the number of surviving individuals. The highest occurrence of lead in water samples was recorded from Žiarske pleso at 2.48µg.l^{-1}. Females and females with eggs demonstrated a greater resistance towards high concentration lead acetate than males. In a parallel test, female L with eggs without lead exposure and females X, Y with 48 hrs. exposure to the lead acetate at 5.5mg.l^{-1} (according to the rate 24EC50), new individuals were developed only by female L, which shows the degenerative effects of lead on reproduction organs and evolution in the next generation. Lead effects were expressed on morphology, too, as a change of the abdominal part of dead individuals compared to individuals without lead acetate exposure. Deformation of the abdominal part was found sometimes to 90°. The effects occured also in the change of the color of the individual, especially in the tests with increased temperature which resulted in the total etiolation and the loss of pigment of the individuals. Based on the PCR, we suspect that the influence of lead as an environmental stressor evokes the morphological changes in the individuals of the monitored species and also the changes in the genome. PCR fragments for the mitochondrial genes regions COI and cytb and 12sRNA occurred in the individuals exposed to the higher concentrations of lead and the higher temperature, while there were no fragments or fragments were found only in the low intensity in the individuals living without the influence of lead. Cyclops abyssorum tatricus seems to be a suitable biological marker for monitoring heavy metal exposure of alpine lakes.

Supported by grant VEGA 1/0336/08/9 by Ministry of Education of SR.

6: Kalafová, A.[1] – Kováčik, J.[1] – Massányi, P.[1] – Schneidgenová, M.[1] – Chrastinová,Ľ. – Jurčík, R.[2] – Lukáč, N.[1] – Chrenek, P.[2] – Čupka, P.[1]:

THE EFFECT OF NICKEL AND ZINC EXPERIMENTAL ADMINISTRATION ON SELECTED HAEMATOLOGY PARAMETERS IN RABBITS

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[2]Animal Production Research Centre Nitra, Slovak Republic]

In this study the effect of nickel and nickel with zinc supplementation on haematological parameters in rabbits, including red blood cell count (RBC), white blood cell count(WBC) and haematocrit value (HCT) were analyzed. Animals were divided into 5 groups: control group K (♀n=5; ♂n=4) and 4 experimental groups P1, P2, P3 and P4 (♀n=5; ♂n=4).Experimental animals received nickel or nickel+zinc in the following amounts: P1 group 17.5 mg $NiCl_2$.kg^{-1}, P2 group 35.0 mg $NiCl_2$.kg^{-1}, P3 group 17.5 mg $NiCl_2$.kg^{-1} + 30 mg $ZnCl_2$.kg^{-1} and P4 group 35 mg $NiCl_2$.kg^{-1} + 30 mg $ZnCl_2$.kg^{-1} added to the feed mixture for 90 days. During this period, blood was collected every month. After the experimental period (90 days), statistically insignificant differences were detected. Our study did not confirm a significant effect of nickel or nickel with zinc on observed haematological parameters in rabbits.

7: Kováčik,A.[1] – Bulla, J.[1] – Trakovická, A.[2] – Rafayová, A.[2] – Lieskovská, Z.[2]:

EFFECT OF CANDIDATE GENES LOCALIZED ON CHROMOSOME 6 OF PIG ON PHYSIOLOGICAL PROCESS OF FAT PRODUCTION

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[2] Department of Genetics and Breeding Biology, Slovak University of Agriculture in Nitra, Slovak Republic]

The aim of this work was to analyze productive traits of pigs following polymorphism of gene *LEPR, MC5R* and *H-FABP*. We observed effect of these genes on physiological process of fat production in pigs. *LEPR (HpaII)* is a candidate gene with effect on fat content. *MC5R (Hsp92I)*

is candidate gene with effect on fat content in pork. It belongs to the melanocorticotropic gene family. *H-FABP(HinfI)* was suggested to be associated with IMF content. These genes substantially impact dietary quality of pork meat. On the basis of tests performed the influence of these candidate genes on intramuscular fat content (IMF) and backfat thickness (BFT) was confirmed. The set of pigs shows double allele polymorphism, but for *LEPR* and *H-FABP* genes we found three genotypic combinations. We found two genotypic combinations for *MC5R* gene. For *MC5R* in genotype AG was found to cause statistically significant lower backfat thickness (AA=10.52 and AG=8.84). On the basis of genotype *LEPR* a high statistical significance was found in BB for BFT as in AB and AA genotypes (AA < AB < BB). On the basis of genotype *H-FABP* differences followed a similar tendency as for BFT. Difference in backfat thickness was highly significant, with the following tendency: AA < AB < BB. Animals with G allele were found to have lower backfat thickness, but this allele has a low incidence. In selection of animals in case of low backfat thickness it is more appropriate to use genotypes *LEPR* and *H-FABP* because B(h) alleles occur in higher frequency.

8: Langrová, T. – Sláma, P.:
INFLUENCE OF *STAPHYLOCOCCUS AUREUS* AND *STREPROCOCCUS UBERIS* ON APOPTOSIS
[Mendel University of Agriculture and Forestry in Brno, Czech Republic]

The aim of this study was to review the influence of the significant bacterial pathogens, *Staphylococcus aureus* and *Streptococcus uberis* on programmed cell death (apoptosis), and to determine the dynamic of morphological changes during aging neutrophil granulocytes of the heifer mammary gland *in vitro*. Using light and electron microscopy, we have noted characteristic alterations of apoptotic neutrophils. These run in three consequential phases. We found that the interaction of bacterial pathogens with neutrophils during incubation leads to expressive quantitative and qualitative changes in apoptotic cells representation. The influence of both pathogens on mammary gland neutrophils caused the deferral of apoptosis expression. Here, *S. aureus* caused lower number of apoptotic neutrophils compared to *S. uberis*. The results demonstrated that *S. aureus* and *S. uberis* interaction with heifer mammary gland neutrophils *in vitro* causes alterations relating to apoptosis of these cells. Looking at the results, we can conclude that the pathogens *S. aureus* and *S. uberis* are not only significant in the heifer mammary gland. They significantly influence the cells of the defensive system in their functions, too. They significantly decrease the appearance of morphological apoptosis manifestations on neutrophils of tissue pool of the heifer mammary gland. The numbers of apoptotic cells in neutrophil population confirm that during the interaction with the aforementioned pathogens, morphological apoptosis manifestations are deferred. Higher numbers of apoptotic neutrophils in stages of apoptotic corpuscles imply the increasing dynamic of this process. Besides that, the dynamic of the apoptotic process is influenced by the specifity of certain bacteria, too.

9: Lauková, M. – Petrák, J. – Vargovič, P. – Križanová, O. – Kvetňanský, R.:
THE EFFECT OF STRESS EXPOSURE ON GENE EXPRESSION OF ADRENERGIC RECEPTORS AND CYTOKINES IN RAT SPLEEN
[Institute of Experimental Endocrinology SAV, Bratislava, Slovak Republic]

Physiological responses induced by stress involve activation of the sympathoadrenal system. Catecholamines (adrenaline, noradrenaline) are the main effectors of stress reaction and mediate their effects through adrenergic receptors (ARs). Immune organs contain rich sympathetic innervation as well as adrenoceptors‘ gene expression. Therefore stress exposure, with a subsequent rise in catecholamine levels, can stimulate alterations in these organs. The aim of our experiment was to investigate changes in gene expression of ARs as well as of several cytokines in the spleen of rats exposed to immobilization stress (IMMO). Male Sprague-Dawley rats of 3 months of age were immobilized once (1x) and repeatedly for 14 consecutive days for 2 h daily and decapitated immediately after termination of the stress stimulus. Spleens were dissected from rats, frozen and used for RNA isolation. Detection of adrenoceptor and cytokine gene expression was performed using RT-PCR. Relative quantification of mRNAs was expressed as a ratio of specific gene mRNA and housekeeping gene (GAPDH) mRNA. We detected the considerable decrease in α2C- and α2A-AR mRNA after repeated (14x) IMMO, but also an increase in α2C-AR after single IMMO. We found a significant rise in mRNA levels of β2-AR in rats exposed to short-term (1x) as well as repeated (14x) IMMO. We also detected the increase in gene expression of pro-inflammatory cytokines (IL-6, IL-18) and a decrease of anti-inflammatory cytokine (IL-10) predominantly after repeated IMMO. Our results suggest different regulation of individual types of adrenoceptors and cytokines mRNA by the stressor. The elevation of β2-AR and a drop in α2-ARs mRNA indicate their predominant function in immune system regulation. The increase of pro-inflammatory and a decrease of anti-inflammatory cytokines point to induction of possible local inflammatory process in the spleen after exposure to stress. Nevertheless, further investigations are needed to explain the exact physiological role of these alterations.

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10: Leska, A. - Dusza, L.:
PROLACTIN AND THYROTROPIN GENE EXPRESSION IN DOMESTIC GANDER PITUITARY GLAND DURING THE SEASON
[Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Poland]

Prolactin (PRL) and thyrotropin (TSH), peptides produced by the anterior lobe of the pituitary gland, play critical roles in the regulation of bird seasonal reproduction. TSH is known to stimulate the thyroid gland to synthetize and release thyroid hormones triiodothyronine (T3) and thyroxine (T4), while PRL acts directly on gonad function. In most birds, the high PRL and T3 and T4 plasma level is essential to evoke and maintain photorefractoriness, the period of lack of reproductive activity. To our knowledge, there is no information concerning PRL and TSH gene expression in the bird pituitary during the season. This study aimed to develop this theme using

male domestic goose (Anser anser f. domestica), the species characterized by the most definite reproductive seasonality within farm poultry. The research was carried out on White Koluda ganders in three characteristic phases of their annual reproductive cycle: the peak of reproductive activity (March, n=7), summer photorefractoriness (July, n=7) and fall sexual reactivation (November, n=7). The PRL and TSH gene expression was analyzed by Real Time PCR using Sybr Green assay (Applied Biosystems). The relative quantification with GAPDH as reference gene was carried out. The PCR reaction specificity was confirmed by the dissociation curves and amplicons sequencing. The PRL and TSH gene expression changed throughout the season. The highest PRL mRNA pituitary content was observed in the non-breeding season (July) as compared to the remaining phases ($p<0.05$). The TSH gene expression was low in the peak of reproduction and the period of its termination, but increased significantly in the fall sexual reactivation phase ($p<0.05$). In summary, the highest PRL transcript production in the period of lack of reproductive activity may support the main role of PRL in the development of photorefractoriness in domestic goose and can be connected with the evolutionary encoded parental behavior. The maximum TSH gene expression in fall may result from intensive metabolism as a consequence of feather repairing after a few induced molting processes, which is common in the poultry industry, and as an effect of recovery of reproductive activity.

11: Lieskovská, Z. - Rafayová, A. - Trakovická, A. – Kováčik, A.:
LEPTIN FUNCTION IN VERTEBRATES
[Slovak University of Agriculture in Nitra, Slovak Republic]

The aim of our work is to summarize the physiological functions of leptin. Leptin is a protein (hormone) produced by adipose tissue cells, which controls the degree of fat deposition and affects appetite and energy expenditure. Leptin, which is the product of the LEP gene, plays a role as a signal of being sated to the hypothalamus. Leptin's concentration decreases with reduced food intake and increases with increased food intake. In vertebrates, the ability to store sufficient quantities of energy-dense triglyceride in adipose tissue allows survival during the frequent periods of food deprivation encountered during evolution. However, the presence of excess adipose tissue can be maladaptive. A complex physiological system has evolved to regulate fuel stores and energybalance at an optimum level. Leptin secreted by adipose tissue and its receptor are integral components of this system. Leptin also signals nutritional status to several other physiological systems and modulates their function.

This work was supported by project VEGA No. 1/4440/07.

12: Lukáč, N.[1] – Massányi, P.[1] – Slamečka, J.[2] – Jurčík, R.[2] – Capcarová, M.[1] – Kalafová, A.[1] – Schneidgenová, M.[1:]
DETERMINATION OF REPRODUCTIVE PARAMETERS OF BROWN HARE *(Lepus europaeus)*
[[1]Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovak Republic;
[2]Animal Production Research Centre Nitra, Slovak Republic]

This study applied the method of coloration of placental scars with potassium hexacyanoferrate to estimate litter size and pregnancy rate during the previous reproductive period. Since the tribal stage of hares decreases rapidly, this method seems to be the ideal way for detecting the quality of reproduction. The results of this study suggest that litter size this year does not differ from last year's, which was high. Hares were obtained in 6 hunting grounds in West Slovakia. We collected young hares in December. The average value was 51.65 %. 2.09 cub ones fall on one female hare per year. However, the result according to placental scars showed an average value of litter size 7.28 for one female hare. With ideal conditions, the average value of young hares would increase from 29% to 79%. Similar results were reached by foreign institutions as well, increasing the validity of this method. Thus, decreasing of tribal stages is a result of environmental deterioration, when young hares do not survive as a consequence of ecological factors. These external factors merit special research and it is necessary to eliminate them.

This work was supported by project VEGA No 1/4347/07.

13: Luptáková, L. – Bálent, P. – Valenčáková, A. – Malčeková, B. – Petrovová, E.:
SEROPREVALENCE OF ANTI-TOXOPLASMA ANTIBODIES IN ANIMALS KEPT IN HOUSEHOLDS
[University of Veterinary Medicine in Košice, Slovak Republic]

This study presents the results of serological examination of household animals. The presence of antibodies against Toxoplasma gondii in household cats, dogs and rabbits was observed by complement fixation test. Blood specimens were collected from 92 asymptomatic animals. Tests were carried out using a complement fixation test where all titres over 1:8 were evaluated as positive. Out of the 92 examined serum specimens titrated at ratios of between 1:8 and 1:64, 47 samples were positive (51.1 %). Of the total number of 32 dog sera, 19 samples (59.4 %) reacted positively and 13 (40.6 %) sera were negative. 13 sera samples out of the total number of 39 cat samples examined reacted positively (33.3 %) and 26 samples reacted negatively (66.7 %). Out of a total of 21 rabbit samples, 15 (71.4 %) reacted positively and 6 (28.6 %) reacted negatively. These results point to the fact that toxoplasmosis is quite wide-spread in household animals.

14: Nestor, C. C. - Seebaugh, A. - Valent, M. - Goodman, R. L. - Hileman, S .M.:
A POTENTIAL ROLE FOR KISSPEPTIN IN PUBERTY ONSET IN SHEEP
[West Virginia University, Morgantown U.S.A]

In peripubertal sheep, a decrease in inhibition by estradiol leads to an increase in gonadotropin releasing hormone (GnRH), and thus luteinizing hormone (LH) secretion, heralding the onset of puberty. Still, the neural mechanisms governing this process remain largely unknown. Kisspeptin, a recently discovered neuropeptide, is expressed in the arcuate nucleus of the hypothalamus of sheep and stimulates GnRH/LH secretion. We hypothesized that kisspeptin expression would be greater in postpubertal as compared to prepubertal sheep and that kisspeptin expression would be increased in the absence of steroid negative feedback. Four groups of females were used: prepubertal intact (n=3), prepubertal ovariectomized (n=4), postpubertal ewes in the early follicular phase (n=3), and postpubertal ovariectomized (n=3). To examine potential sex differences in kisspeptin expression, 4 groups of males

(n=3 to 4 per group) which were similar in age to the females and either intact or castrated were also used. Blood samples were collected via jugular venipuncture at 12-min intervals for 4 hours prior to sacrifice, after which brains were perfused with 4% paraformaldehyde and hypothalami collected for immunoctyochemical evaluation of kisspeptin expression. LH was measured to confirm the endocrine state of each animal. Mean levels of LH and LH pulse frequency were suppressed by the presence of the gonads in both males and females. In females, kisspeptin expression was greater in postpubertal ewes as compared to prepubertal ewes, and was increased by ovariectomy only in the prepubertal group. In the males, cell numbers did not differ with age, but were increased by castration in both age groups. Overall, females had a significantly higher number of kisspeptin-expressing cells in comparison to males. The increase in kisspeptin cell numbers in postpubertal females is consistent with a role for kisspeptin in puberty onset in the sheep. Further, the increase in kisspeptin expression with ovariectomy in prepubertal females indicates that decreased kisspeptin cell numbers in intact prepubertal females is due to the negative feedback effects of estradiol. The increase in kisspeptin expression in males with castration indicates that gonadal steroids regulate kisspeptin. Further, since ram lambs undergo puberty much earlier than ewe lambs, the lack of age differences in the males suggests that differences in the females relate to puberty rather than age.

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15: Nitkiewicz, A. - Smolinska, N. - Przala, J. – Kaminski, T.:
OREXIN TYPE 1 RECEPTOR PROTEIN EXPRESSION IN THE PORCINE OVARY DURING THE OESTOUS CYCLE
[University of Warmia and Mazury in Olsztyn, Poland]

Orexins are novel neuropeptides which are synthesized by proteolytic processing from a single precursor named prepro-orexin, constructed from 130 amino acids. Both orexins act through specific G protein-coupled protein receptors termed orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R). The widespread distribution of the orexins and their receptors suggests that these peptides may play an important role in several physiological functions, including feeding behaviour, energy homeostasis, sleep and wakefulness, as well as the reproductive system. To our knowledge there is no data concerning OX1R protein expression in porcine ovary during the oestrous cycle. Therefore, the aim of the study was the comparison of OX1R protein content in corpora lutea on days 2-3, 10-12 and 14-16 of the oestrous cycle and in granulosa and theca interna cells during days 17-19 of the cycle, using Western blot analysis. The protein samples were electrophoresed on a 12.5% SDS polyacrylamide gel, separately for OX1R and actin, and then transferred to nitrocellulose membranes. After blocking in TBST with 5% skimmed milk, membranes were incubated overnight with sheep polyclonal antibodies for OX1R at a dilution 1:100 or rabbit polyclonal antibodies for actin at a dilution 1:200, which were used as a internal control. After washing in TBST buffer, membranes were transferred for 1.5 hour to secondary rabbit anti-sheep IgG antibodies for OX1R (diluted 1:500), or goat anti-rabbit IgG for actin (diluted 1:5000) conjugated with alkaline phosphatase. As a detector of immunocomplex was used NBT/BCIP system. The results of Western blot were quantified by densitometric scanning of immunoblots with GelScan for Windows ver. 1.45 software. Data were expressed as a ratio of OX1R protein relative to actin protein in arbitrary optical density units. It was shown for the first time OX1R protein expression in porcine ovary during the oestrous cycle. OX1R protein concentration was higher in corpora lutea ($p<0.01$) on days 2-3, 10-12 and 14-16 than in granulosa and theca cells from 17-19 day of the oestrous cycle. Moreover, on days 2-3 the OX1R protein was higher ($p<0.05$) compared with days 10-12. The greatest OX1R protein was observed on days 2-3 of the oestrous cycle. In conclusion, sensitivity of ovaries to orexins changes during the oestrous cycle. Moreover, diversified OX1R protein expression may suggest that orexins play an important role in the reproductive system of the pig.

16: Okuliarová, M. - Škrobánek, P. – Zeman, M.:
SELECTION ON TESTOSTERONE CONCENTRATIONS IN THE EGG YOLK AS A MODEL TO STUDY HORMONE-MEDIATED MATERNAL EFFECTS IN BIRDS
[Faculty of Natural Sciences, Comenius University Bratislava, Slovak Republic]

Epigenetic maternal effects mediated by deposition of hormones into the egg yolk can exert profound consequences on offspring development. The most frequent approach to study hormone-mediated maternal effects is using experimentally increased testosterone content in the egg yolk. In our previous experiments, we found distinct differences among female Japanese quail in concentrations of yolk testosterone. The results led to a prediction that the variability in deposition of this androgen into the yolk can be at least partially explained by genetic variation among mothers. From a random-bred population of Japanese quail we selected females laying eggs with extremely high and extremely low yolk testosterone concentrations, respectively. These birds formed the parental generation for divergent selection, which resulted in two lines of Japanese quail with high (HET) and low (LET) egg testosterone content. As predicted, significant differences between these lines in yolk testosterone concentrations were found in both the F1 and F2 generation. This selection approach allows estimation of the genetic heritability of maternal hormone deposition for the first time. Moreover, it opens interesting possibilities to study the effects of increased maternal testosterone levels on behavioural and physiological phenotype of offspring.

17: Rafayová, A. - Lieskovská, Z. - Trakovická, A. - Parkányi, V. – Kováčik, A.:
EFFECT OF SUPERANALOG GnRH ON REPRODUCTIVE CYCLE OF RABBITS
[Slovak University of Agriculture in Nitra, Slovak Republic]

The aim of this study was to optimise intravaginal application of superanalog GnRH to positively affect induction of ovulation and conception rate. A control group of females were given an intramuscular application of GnRH (2.5μg/I.D.). Females from the experimental group were given an intravaginal application of superanalog GnRH – Lecirelinum (Group S1 – 2.5 μg/I.D., Group S2 – 5.0 μg/I.D., Group S3 – 7.5 μg/I.D., Group S4 - 15 μg I.D. The conception rate of the control group was 62.74 ±13.70. The conception rates of the experimental group were: S1 = 24.99 ± 5.64, S2 = 59.97 ± 11.55, S23 = 72.09 ± 2.96 and S4 = 52.77 ± 3.92. Significant differences

were found between females from group S1 and females from groups S2, S3, and control. It follows that intravaginal dosage in Group S1 is insufficient for ovulation induction and sperm-egg interaction. Intravaginal application of superanalog GnRH in quantity 7.5 µg/I.D positively affects ovulation induction with the benefit (+9.35%) of increasing conception rate (CR = 72.09 ± 2.96).

18: Sirotkin, A.V. - Grosmann, R. – Rafay, J.:
INVOLVEMENT OF METABOLIC HORMONES LEPTIN, GHRELIN AND OBESTATIN IN CONTROL OF OVARIAN CELL FUNCTIONS
[Animal Production Research Centre Nitra, Slovak Republic]

Nutrition can control reproductive processes via changes in production of metabolism-dependent hormones leptin, ghrelin and obestatin. The aim of the present study was to examine the role of these hormones in control of basic ovarian cell functions (proliferation, apoptosis, secretory activity) in different species (rabbit, pig and chicken). It was observed that nutritional patterns can affect the plasma level of some metabolic hormones, as well as change animal and avian reproductive parameters, basic functions of ovarian cells and their response to physiological stimulators (gonadotropins a.o.). On the other hand, treatment of animals or their isolated ovarian cells with metabolic hormones were able to change ovarian cell proliferation, apoptosis, release of reproductive hormones, oocyte maturation and ovulation rate. Treatments of cells with pharmacological blockers of protein kinase A, MAP kinase and CDC2 kinase prevented or inverted effects of leptin and ghrelin on ovarian cells. The present observations suggest that nutrition can control ovarian functions via changes in release of metabolic hormones (leptin, ghrelin, obestatin), which regulate basic ovarian cell functions (proliferation, apoptosis, secretory activity, oogenesis and ovulation) through protein kinases-dependent intracellular mechanisms.

19: Slivková, J. - Roychoudhury, S. – Massanyi, P.:
TIME-DEPENDENT MOTILITY OF RABBIT SPERMATOZOA EVALUATED BY A COMPUTER AUTOMATED SEMEN ANALYSER – CASA
[Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovak Republic]

Visual-motility assessment is a tool used to determine the quality of various ejaculates. This method is subjective, and consequently, computer automated semen analysis (CASA), with different software designs, has been developed for more objective assessment than using conventional image analysis or particle counting. In our study, a commercially available computer automated semen analyzer (Sperm Vision™; Minitüb – Germany) was used for evaluation of parameters of spermatozoa motility in 15 rabbit ejaculates. The purpose of this study was to determine the time-dependent (0, 60 and 120 minutes) variations in the observed parameters. The highest percentage of motility was at time 0 (80.44 %). At 60 min, the percentage of motil sperm was 79.92 %, and at 120 min, it was 71.15 %. Similarly, progressive motility at time 0 was highest at 70.72 %; at time 60 min it was 63.40 %; and at time 120 min it was 55.41 %. Also, other distance and velocity motility parameters showed time-dependent variation. Data from this study determines general time-dependent tendencies of spermatozoa motility in rabbits.

20: Smolinska, N. - Nitkiewicz, A. - Przala, J. – Kaminski, T.:
OREXIN TYPE 1 RECEPTOR PROTEIN EXPRESSION IN THE PORCINE PITUITARY GLAND DURING THE OESTROUS CYCLE
[Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Poland]

Orexin A and B are hypothalamic peptides derived from a precursor called prepro-orexin and associated with the stimulation of food intake and arousal. These peptides bind and activate two G protein-coupled receptors, termed orexin receptor 1 (OX1R) and orexin receptor 2 (OX2R). OX1R is selective for orexin A, whereas OX2R binds both orexins with similar affinity. There is evidence indicating that orexins act on some pituitary functions and their receptors fluctuate throughout the oestrous cycle. Most results concerning the orexin system in the pituitary have been obtained from studies on rodents and humans. Therefore, the purpose of the present study was to compare the expression levels of OX1R protein by Western blotting in anterior (AP) and posterior pituitary (NP) during days 2-3, 10-12, 14-16, and 17-19 of the oestrous cycle. Denatured protein preparations extracted from the porcine AP and NP, were resolved by SDS-PAGE (12.5%) and transferred to nitrocellulose membranes. Membranes were blocked for 5 h in TBST containing 5% skimmed milk powder, then overnight with sheep polyclonal antibodies to OX1R at a dilution of 1:100 or rabbit polyclonal antibodies to actin at a dilution of 1:200, which were used as an internal control for equal loading as well as to quantify porcine OX1R proteins. To identify immunoreactive bands, membranes were incubated for 1.5 h with rabbit anti-sheep IgG for OX1R (diluted 1:500) or goat anti-rabbit IgG for actin (diluted 1:5000) conjugated with alkaline phosphatase. Nonspecific foetal calf serum was used instead of primary antibodies to produce negative control blots. The immunocomplexes were visualized using NBT and BCIP. The results of Western blotting were quantified by densitometric scanning of immunoblots with GelScan for Windows ver. 1.45 software. Data were expressed as a ratio of OX1R protein relative to actin protein in arbitrary optical density units. OX1R protein in AP was the greatest ($p<0.05$) on days 10-12 of the oestrous cycle, while the lowest ($p<0.05$) during the follicular phase of the cycle. In NP, OX1R protein was most abundant on days 2-3 and 14-16 compared to days 10-12 and 17-19 of the cycle ($p<0.05$), but the lowest on days 17-19 ($p<0.05$). Expression of OX1R protein was higher ($p<0.001$) in AP than NP on days 10-12 of the cycle. In conclusion, our results might suggest that orexins can participate in the control of pig reproduction by exercising their action at the pituitary level and have a direct effect on these organs during the oestrous cycle. Moreover, changes in OX1R protein expression in the porcine pituitary strongly suggest that its sensitivity to orexins varies throughout the oestrous cycle.

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21: Škrobánek, P. - Baranovská, M. - Šárniková, B. - Zeman, M. – Almášiová, V.:
EFFECT OF SIMULATED WEIGHTLESSNESS ON MALE JAPANESE QUAIL REPRODUCTION
[Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences,Ivanka pri Dunaji, Slovak Republic]

Hypodynamia can be used to simulate weightlessness

in the laboratory conditions. The objective of our study was to investigate the effects of chronic hypodynamia on the growth and development of the testes and cloacal gland, and plasma testosterone concentration in Japanese quail. The testis weight in males reared under hypodynamia was significantly lower compared to age-matched control between 21 and 63 days of age ($P<0.05$). The cloacal gland area of experimental birds calculated from its width and length was also smaller in comparison with control quail from 35 to 56 days of age ($P<0.05$). The foam production was significantly lower in hypodynamia males at age 35, 42, 49 and 63 days ($P<0.05$). The level of plasma testosterone concentration was significantly reduced in hypodynamia birds between 35 and 70 days of age ($P<0.05$), with the exception of day 56. These results provide further evidence that although hypodynamia negatively affects the examined variables, the male Japanese quail is able to develop normally under conditions of simulated weightlessness.

22: Turanová, Z.[1,2] – Pivko, J.[1,2] – Chrenek, P.[1,2]:
ULTRASTRUCTURE OF TRANSGENIC RABBIT EMBRYOS
[[1]Animal Production Reserch Centre, Nitra, Slovak Republic; [2]Department of Animal Physiology, Slovak University of Agriculture in Nitra, Slovak Republic]

The objective of this study was to investigate the ultrastructure (relative volume) of normal cell components of transgenic rabbit embryos (vacuoles, dense bodies, cellular debris, lipids, floculent vesicules) and cell components, which allocated pathological changes at the ultrastructural level (cytoplasmatic envelope, nuclear envelope, trophoblastic microvilli, junctional contacts, mitochondria, rough endoplasmatic reticulum, Golgi apparatus). Transgenic rabbit embryos were obtained by microinjection of EGFP gene (enhanced green fluorescent protein). We observed 3 groups of rabbit embryos: intact group, transgenic – EGFP positive and non-transgenic – EGFP negative group. The second and the third group were vitrified in liquid nitrogen and subsequently thawed. Ultrastructure was analysed using electron microscopy of Durcupan ACM thin sections of rabbit embryos. In case vacuoles, cellular debris, floculent vesicules, nuclear envelope and junctional contacts significant differences ($p<0.01$) between non – transgenic and control group, and between non – transgenic and transgenic group of rabbit embryos 3: $(1, 2)^{**}$ were found. Significant differences ($p<0.05$) was obtained in case of cytoplasmic envelope, trophobalstic microvilli and mitochondria between control and transgenic embryos and between control and non – transgenic group 1: $(2, 3)^{*}$. Mitochondria shown a significant difference ($p<0.05$) in comparison of transgenic to non – transgenic group 2: 3^{*}. We also recognized significant difference ($p<0.01$) in case of lipids and rough endoplasmic reticulum in comparison transgenic group to control, and transgenic to non – transgenic group of rabbit embryos 2: $(1, 3)^{**}$. Our results show that integration of transgene and vitrification may cause pathological changes in ultrastructure of transgenic rabbit embryos.

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Thank to Ing. Pavel Fľak DrSc., for help with statistical analyses.

23: Valent, M. – Hardy, S. L. – Bogusz, A. L. – Sigh, S. R. – Hileman, S. M. – Billings, H. J. – Nestor, C. C. – Holaskova, I. – Connors, J. M. – Goodman, R. L.:
ROLE OF A15 DOPAMINE NEURONS IN THE SEASONAL REPRODUCTION OF SHEEP
[Department of Physiology and Pharmacology, West Virginia University, Morgantown, U.S.A]

Most breeds of ewes express reproductive cycles in the fall and winter, but are anovulatory during the spring and summer (i.e. anestrus). This pattern of reproduction is caused by seasonal changes in response to estradiol negative feedback: during the breeding season estradiol produces only a slight suppression of LH secretion, but during anestrus it is a potent inhibitory steroid. Our laboratory is investigating the changes in hypothalamic function responsible for this seasonal variation in estradiol negative feedback. We, together with our colleague, Jean-Claude Thiery, identified a group of dopaminergic neurons (A15 DA) just posterior to the optic chiasm that play a key role in seasonal reproduction. Several lines of evidence indicate that these neurons are stimulated by estradiol to inhibit GnRH secretion in anestrus, but do not respond to this steroid in the breeding season. Our data demonstrate that estrogen increases multiunit electrical activity (MUA) in the A15 region of ewes during inhibitory photoperiod and thus inhibit GnRH secretion. Local administration of crystalline estradiol into A15 area result in the stimulation of A15 DA neurons, which in turn suppresses LH secretion in anestrous ewes. However, A15 neurons do not appear to contain estradiol receptors (ER). Therefore, we focused on possible estrogen-responsive afferents to A15 neurons and have identified such neurons in two anatomical areas: the ventromedial preoptic area and the retrochiasmatic area. Neurons in these areas that contain ER project to the A15 and local administration of estradiol in each area inhibits LH secretion via a dopaminergic system. More recently, we have obtained evidence that A15 neurons are regulated by inhibitory GABAergic and stimulatory glutamatergic neural afferents in anestrus. GABAergic neurons contain ER and DA neurons in A15 area express GABA receptors. Estradiol inhibits the GABAergic tone and stimulates glutamate release thus stimulating A15 neurons in anestrus. Additionaly, there are fewer glutamatergic synapses on A15 neurons in the breeding season, raising the possibility that this anatomical change may account in part for the inability of estradiol to stimulate A15 neurons at this time of year.

24: Vargovič P. - Petrák J. - Lauková M. - Kvetňanský R.:
EFFECT OF COLD AND IMMOBILIZATION STRESS ON CATECHOLAMINE levels in rat adipose tissues
[Institute of Experimental Endocrinology SAV, Bratislava, Slovak Republic]

Adipose tissues are organs with dense sympathetic innervation and contain high levels of norepinephrine (NE) and lower levels of epinephrine (EPI). We measured NE and EPI levels in five types of adipose tissue (mesenteric, retroperitoneal, subcutaneous, epididymal and interscapular brown) of rats exposed to immobilization and cold stress. Male Sprague-Dawley rats of 3 months of age were immobilized once (1x) or repeatedly for 14 consecutive days for 2 hours daily and decapitated immediately after termination of the stress stimulus. Another group of rats were exposed to acute (24 h) or chronic (14 days) cold (5°C). Furthermore, rats adapted to

immobilization stress (homotypic stressor) were subsequently exposed to cold stress, which represented the novel (heterotypic) stressor. Adipose tissues were dissected from rats, frozen and used for catecholamine determination by radioimmunoassay. Single and repeated immobilization increased NE end EPI levels in mesenteric, brown and subcutaneous adipose tissue. Exposure to cold increased EPI level in mesenteric, epididymal and interscapular brown adipose tissue. In contrast, NA levels, which are usually much higher, increased only in interscapular brown adipose tissue. Exposure to the novel stressor induced significant increase of EPI level in subcutaneous adipose tissue and NE level in interscapular brown adipose tissue. In summary, stress exposure evoked different changes in catecholamine levels in various types of rat adipose tissue. The obtained data contributes to the understanding of potential functional differencies of the adipose tissues in stress situations.

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25: Vdoviaková, K. – Maženský, D. – Petrovová, E.:
THE BLOOD SUPPLY OF THE TONGUE IN THE RATS
[Univerzity of Veterinary Medicine in Košice, Slovak Republic]

The aim of this study was to investigate the blood supply of the tongue in rats. In this study, we used rats (Rattus norvegicus f. domestica) of the Wistar breed of both sexes. They were euthanized by prolonged anaesthesia induced by aether. We opened the left chamber and scoured whole body with physiological solution. We studied corrosive casts of the circulatory system using Duracryl Dental®. Afterwards, we put the whole body into lukewarm water for polymerisation. Maceration of soft tissue was made in a solution of KOH (2 – 4 %), at a temperature of 60 - 70°C, over 2-3 days. The results of our study conclude that the following arteries participate in blood supply of the tongue in rats: a. lingualis, a. profunda linguae and rr. dorsales linguae. A. lingualis was the branch of the a. carotis externa. In 17 %, the artery originated with truncus linguofacialis; in 83 % together with a. maxillaris and a. facialis. The a. profunda linguae was the main continuation of a. lingualis inside the tongue. Rr. dorsales linguae originated from a. profunda linguae in the dorsal direction to the dorsum linguae. We found 15 to 20 rr. dorsales linguae.

26: Wojciechowicz, B -, Franczak, A. - Żmijewska, A. - Kurowicka, B. – Kotwica, G.:
EFFECT OF INTERLEUKIN 1B (IL-1B) AND INTERLEUKIN 6 (IL-6) ON 17B-ESTRADIOL SECRETION FROM THE PORCINE UTERUS
[University of Warmia and Mazury in Olsztyn, Poland]

The origin of high amounts of 17β-estradiol (E2) present in the porcine uterus during early pregnancy is still under investigation. Our previous results provided evidence that the endometrium and the myometrium can both be a rich source of E2 during early pregnancy and the estrous cycle. In this study, we examined the influence of certain cytokines (IL-1β and IL-6) produced by embryos on steroidogenesis during days 14 - 16 of pregnancy when conceptus estrogen secretion wanes. Slices of endometrium and myometrium (200 - 210 mg) were isolated from uteri of post pubertal pigs (n = 4) on days 15 - 16 of pregnancy. The isolated slices were pre-incubated in 2 ml of M199 medium in an atmosphere of 95 % O_2 + 5 % CO_2 at 37 ^oC. Samples were placed in fresh medium and incubated for the next 6 or 12 hours with treatments: IL-1β and IL-6 in doses of 1 $ng.ml^{-1}$ and 10 $ng.ml^{-1}$. After each incubation, the culture medium was collected and E2 concentration was determined by radioimmunoassay. Concentrations of E2 secretion were compared with ANOVA (Fisher's LSD test). Basal secretion of E2 did not differ either within endometrium and myometrium or between different incubation periods (6 hours vs. 12 hours). After 6 hours of incubation, IL-1β at 1 $ng.ml^{-1}$ and IL-6 at both levels increased secretion of E2 from the endometrium ($p<0.05$). After 6 hours of incubation, both cytokines in both applications increased secretion of E2 ($p<0.05$; in case of 10 ng/ml IL-1 β tendency – $p<0.07$) in myometrium. After 12 hours of incubation, only IL-6 (10 $ng.ml^{-1}$) increased secretion of E2 from the endometrium ($p<0.05$) while IL-1β and IL-6 in both doses stimulated E2 release from the myometrium ($p < 0.05$). In conclusion: 1) the steroid biosynthesis pathway is active in the porcine uterus; 2) Cytokines may play a potential role in uterine steroidogenesis regulation during early pregnancy. Consequently, cytokines derived from the porcine conceptus cannot be neglected in broad spectrum consideration of early pregnancy regulations in pigs.

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27: Wylot, B. - Tworus, K. – Okrasa, S.:
EXPRESSION OF GENES CODING FOR POMC, PENK AND β-LH SUBUNIT IN VITRO BY ANTERIOR PITUITARY CELLS OF CYCLIC GILTS AFTER TREATMENT WITH GnRH, CRH, INHIBIN, ACTIVIN AND OVARIAN STEROIDS
[Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Poland]

Previous data suggest that endogenous opioid peptides (EOP), deriving from proopiomelanocortin (POMC) and proenkephalin (PENK) are involved in the modulation of LH secretion in females. Apart from relatively well-known action of EOP on LH secretion at the hypothalamic level, through the GnRH system, they may also locally modulate this gonadotropin release from the pituitary gland. Our recent studies have demonstrated changes in the expression of genes coding for opioid precursors in porcine anterior pituitary across the estrous cycle. However, data concerning the factors regulating the activity of pituitary opioid systems are limited. Therefore, the aim of the present study was to examine in vitro effects of GnRH, CRH, inhibin, activin and ovarian steroids on the expression of genes coding for POMC, PENK and β-LH subunit in anterior pituitary cells of cyclic gilts. Anterior pituitaries (N=7) were collected from gilts on days 8-10 (luteal phase) and 19-20 (follicular phase) of the estrous cycle. Isolated cells were incubated with GnRH (100 $ng.ml^{-1}$), CRH (10-8 and 10-7M), inhibin (5 and 10 $ng.ml^{-1}$), activin (5 and 10 $ng.ml^{-1}$) and progesterone (30 $ng.ml^{-1}$; only the luteal-phase pituitary cells) or estradiol (25 and 50 $pg.ml^{-1}$; only the follicular-phase pituitary cells). The gene expression was determined by a semi-quantitative Real-Time RT-PCR assay with the use of SybrGreen as a product detection system. GnRH decreased ($p<0.05$) POMC gene expression in the luteal-phase pituitary cells, while CRH (10-8 and 10-7M) and estradiol (50 $pg.ml^{-1}$) increased POMC gene expression in the follicular-phase cells. Expression of PENK gene was reduced in the luteal-phase cells

treated with activin (5 ng.ml^{-1}) and it was elevated in the cells representing follicular phase, treated with CRH (10-8M). The follicular-phase pituitary cells showed reduced expression of β-LH subunit gene after treatment with CRH (10-8 and 10-7M) and activin (5 ng.ml^{-1}). Expression of β-LH subunit gene in pituitary cells remained unchanged following treatment with GnRH, however GnRH significantly increased secretion of LH by these cells. These results indicate that hypothalamic and ovarian factors, tested herein, may influence the expression of genes coding for β-LH subunit as well as opioid peptide precursors (POMC and PENK) in anterior pituitary cells of gilts during different stages of the estrous cycle.

28: Zeman, M.[1] - Molčan, Ľ.[1], Veselá, A.[1] - Blažíček, P.[2] – Herichová, I.[1]:

IS CHRONODISRUPTION RELATED TO HYPERTENSION DEVELOPMENT IN SHIFT WORKERS?

[[1]Department of Animal Physiology and Ethology, Comenius University Bratislava; [2]Alpha Medical a.s., Bratislava, Slovak Republic]

In our experiment, we exposed rats to a shifted light (L)/dark (D) regimen simulating shift work. Blood pressure and heart rate were measured with a tail-cuff plethysmography method or telemetrically on freely moving animals. Hormones (melatonin, thyroxine, triiodothyronine, corticosterone) and metabolites (glucose, uric acid, creatinine, triacylglycerols, cholesterol and phospholipids) were measured in plasma. We did not detect an increase in BP when rats were kept on shifted L/D regimen without additional stressful stimuli. However, their response to stressors may be compromised on the shifted L/D. We concluded that compromised coping with stressors under shift-work regimens may be responsible for higher frequencies of cardiovascular diseases in shift-workers.

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29: Żmijewska, A. - Franczak, A. - Kurowicka, B. - Wojciechowicz, B.- Kotwica, G.:

EFFECT OF CYTOKINES ON PGE2 SYNTHESIS AND SECRETION BY CORPORA LUTEA OF CYCLIC PIGS

[University of Warmia and Mazury in Olsztyn, Poland]

Cytokines, acting as a paracrine factors, may be involved in the regulation of porcine corpus luteum (CL) lifespan during estrous cycle. One of the local luteotrophic mechanism is PGE2 secretion by CL. Thus, the aims of the study were to determine the effect of cytokines on PGE2 synthesis and secretion by porcine cyclic CL. Post pubertal pigs (n=14) were used on days 10-11, 12-13 and 15-16 of the estrous cycle. The isolated slices of CL (100 mg) were pre-incubated in 2 ml M199 medium in atmosphere 95%O_2+5%CO_2 at 37^{0}C. After this time the medium was changed to a fresh one and slices were incubated by the next 6 or 12h with treatments: IL-1β, TNFα and IL-6, all in dose of 10 ng.ml^{-1}. After each time point of incubation, the medium was collected and concentration of PGE2 was measured with ELISA. Subsequently, the slices were used for estimation of mRNA PGE2 synthase (PGEs) expression with real-time PCR. Basal concentrations of PGE2 in medium were higher ($p<0.05$) on days 10-11 and 12-13 than on days 15-16 of the estrous cycle, after 6 and 12h-lasted CL cultures. Interleukin 1β increased ($p<0.05$) PGE2 secretion from CL during all studied days of the estrous cycle. The stimulatory effect of TNFα on PGE2 secretion was observed only after 6h-lasted incubation of CL harvested on days 12-13 the estrous cycle. IL-6 had no effect on PGE2 secretion by cultured CL during all studied days of the estrous cycle. PGEs expression was observed in porcine cyclic CL. In conclusion: 1. Basal synthesis and secretion of PGE2 by porcine CL decreased during luteolysis (days 15-16 of the estrous cycle); 2) IL-1β had a luteotrophic influence on porcine CL by stimulation of PGE2 secretion. Thus, cytokines may be involved in luteal secretion of PGE2.

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