

SESSION XI
PHYSIOLOGY OF REPRODUCTION

Thursday (September 16, 2021; 9:00 – 15:25)

Chair:

Prof. Anita Franczak

Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Prof. Urszula Kosior-Korzecka

Sub-Department of Pathophysiology, Department of Preclinical Veterinary Sciences,
Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland

DETAILED SESSION XI SCHEDULE

Opening lecture (Thursday, September 16, 2021; 9:00 – 9:40; *virtual stream B*)

- S11.L1 EPIGENETIC MECHANISMS AND BIO-MECHANICAL CUES DRIVE CELL DIFFERENTIATION. **T.A.L. Brevini, G. Pennarossa, R. Pasquariello, F. Gandolfi** (Laboratory of Biomedical Embryology, Centre for Stem Cell Research, UniStem, Università degli Studi di Milano, Milano, Italy).

Oral presentations (Thursday, September 16, 2021; 9:40 – 13:25; *virtual stream B*)

- S11.L2 PRESENCE OF LACTOBACILLI WITHIN THE BOVINE FEMALE REPRODUCTIVE TRACT AND THEIR POSSIBLE ROLE DURING REPRODUCTIVE EVENTS. **C. Gabler** (Institute of Veterinary Biochemistry, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany).
- S11.L3 THE IMPACT OF ENERGY METABOLISM CHANGE IN PORCINE CUMULUS-OOCYTE COMPLEX DURING *IN VITRO* MATURATION. **G. Gorczyca¹, K. Wartalski², M. Duda¹** (¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland, ²Department of Histology, Jagiellonian University Medical College, Krakow, Poland).
- S11.L4 SEX STEROID RECEPTOR AGONISTS AND ANTAGONISTS AFFECT THE EXPRESSION OF TRANSCRIPTION FACTOR FORKHEAD L2 (FOXL2) IN THE NEONATAL PORCINE OVARY. **P. Witek, N. Marek, M. Grzesiak, M. Slomczynska, K. Knapczyk-Stwora** (Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland).
- S11.L5 PRO- APOPTOTIC EFFECT OF VASPIN ON HUMAN PLACENTA BeWo CELLS. **M. Dawid, E. Mlyczynska, P. Kurowska, M. Jurek, A. Rak** (Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland).
- S11.L6 RELATIVE ABUNDANCE OF APELIN RECEPTOR TRANSCRIPT IN THE PORCINE PITUITARY DURING THE ESTROUS CYCLE AND EARLY PREGNANCY. **K. Kisielewska, E. Rytelawska, M. Gudelska, M. Kiezun, K. Dobrzyn, E. Zaobidna, K. Bors, G. Kopij, K. Szymanska, B. Kaminska, N. Smolinska, T. Kaminski** (University of Warmia and Mazury, Olsztyn, Poland).
- S11.L7 VISFATIN/NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) EXPRESSION IN PORCINE CORPUS LUTEUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY. EFFECT OF LUTEINIZING HORMONE (LH) AND P₄ ON VISFATIN PROTEIN LEVEL. **E. Mlyczynska¹, E. Zaobidna², E. Rytelawska², M. Kiezun², K. Dobrzyn², N. Smolinska², T. Kaminski², A. Rak¹** (¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo, Poland).
- S11.L8 CHEMERIN AS A HORMONE MODULATING ENDOMETRIAL REMODELING IN PIGS DURING THE PERI-IMPLANTATION PERIOD: AN *IN VITRO* STUDY. **E. Rytelawska, M. Kiezun, K. Dobrzyn, E. Zaobidna, M. Gudelska, K. Kisielewska, K. Bors, G. Kopij, K. Szymanska, B. Kaminska, T. Kaminski, N. Smolinska** (University of Warmia and Mazury, Olsztyn, Poland).
- S11.L9 DECORIN AND DERMATOPONTIN DIFFERENTIALLY AFFECT CARUNCULAR EPITHELIAL CELL ADHESION IN PREGNANT COWS. **M. Jamiol¹, J. Wawrzykowski¹, M. Kankofer¹** (¹Department of Biochemistry, Faculty of Veterinary Medicine, University of Life Science in Lublin, Lublin, Poland).
- S11.L10 THE ELECTROMAGNETIC FIELD (EMF) RADIATION INDUCES TRANSCRIPTOMIC ALTERATIONS IN PIG MYOMETRIUM DURING THE PERI-IMPLANTATION PERIOD. **E.M. Drzewiecka¹, W. Kozłowska¹, L.P. Pauksto², A.Z. Zmijewska¹, P.J. Wydorski¹, J.P. Jastrzebski², A. Franczak¹** (¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland, ²Department of Plant Physiology, Genetics and Biotechnology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).
- S11.L11 PPAR_γ REGULATES THE EXPRESSION OF GENES IN THE PORCINE INFLAMED ENDOMETRIUM DURING FOLLICULAR PHASE OF THE ESTROUS CYCLE. **K. Mierzejewski¹, L. Pauksto², A. Kurzynska¹, Z. Kunicka¹, J.P. Jastrzebski², M. Golubska¹, I. Bogacka¹** (¹University of Warmia and Mazury in Olsztyn, Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology; Olsztyn, Poland, ²University of Warmia and Mazury in Olsztyn, Faculty of Biology and Biotechnology, Department of Plant Physiology, Genetics and Biotechnology, Olsztyn, Poland).
- S11.L12 KISS-1/GPR54 mRNA EXPRESSION AND THE RELATIONSHIP BETWEEN KISS-10 AND LUTEINIZING HORMONE SECRETION IN PITUITARY GLAND OF CYCLIC AND PCOS - AFFECTED SOWS. **U. Kosior-Korzecka¹, V. Longo², C. Della Croce², N. Szyslak¹, A. Furmanczyk-Gnyp¹, A. Nowakiewicz¹, I. Puzio³, B. Surowka¹, N. Minakow¹, B. Szymczak¹** (¹Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland, ²National Research Council, Institute of Agricultural Biology and Biotechnology, Pisa, Italy, ³Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland).
- S11.L13 KISS-INDUCED ALTERNATIONS IN PRL mRNA TRANSCRIPT ABUNDANCE IN PORCINE PITUITARY CELLS DURING THE ESTROUS CYCLE. **A. Zmijewska¹, W. Czelejewska^{1,2}, E.M. Drzewiecka¹, S. Okrasa¹, A. Franczak¹** (¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, ²Department of Neurosurgery Laboratory of Regenerative Medicine, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).

- S11.L14 EFFECTS OF LONG-TERM CHANGES IN BODY WEIGHT ON THE ABILITY OF RESISTIN TO MODULATE REPRODUCTIVE HORMONES IN SHEEP. **M. Szczesna, W. Biernat, K. Kirsz, D.A. Zieba** (Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Sciences, University of Agriculture in Krakow, Krakow, Poland).
- S11.L15 VASPIN ENHANCED PORCINE OOCYTES *IN VITRO* MATURATION *VIA* MAP3/1 AND PRKAA1 KINASES PATHWAYS. **P. Kurowska¹, M. Mlyczynska¹, A. Estienne², A. Barbe², I. Rajska³, K. Sobol³, K. Poniedzialek-Kempny³, J. Dupont², A. Rak¹** (¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland, ²INRAE, UMR85, Unite Physiologie de la Reproduction et des Comportements, Nouzilly, France, ³ Department of Reproductive Biotechnology and Cryopreservation, National Research Institute of Animal Production, Balice, Poland).

Session summary

Poster session (Thursday, September 16, 2021; 13:30 – 15:25; *virtual stream C*)

- S11.P1 ELECTROMAGNETIC FIELD OF EXTREMELY LOW FREQUENCY INDUCES CHANGES IN THE RELATIVE ABUNDANCE OF *HSD17B2* AND *VDR* IN THE ENDOMETRIUM OF PIGS DURING THE PERI-IMPLANTATION PERIOD. **W. Kozłowska, E.M. Drzewiecka, A. Zmijewska, P.J. Wydorski, A. Franczak** (Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).
- S11.P2 EFFECT OF VITAMIN D3 ADMINISTRATION ON GLUCOSE AND INSULIN LEVEL, AND HOMA-IR INDEX IN RATS WITH LETROZOLE-INDUCED PCOS. **M. Grzesiak¹, K. Kaminska¹, O. Fraczek¹, A. Szlaga², A. Maslanka³, D. Klimczyk³, P. Sambak², P. Dziurawicz¹, K. Knapczyk-Stwora¹, A. Blasiak², A. Rak³** (¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ³Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland).
- S11.P3 PROTEIN EXPRESSION AND IMMUNOLocalISATION OF VASPIN AND GRP78 RECEPTOR IN HUMAN PLACENTA OF INTRAUTERINE GROWTH RESTRICTION. PRELIMINARY DATA. **M. Jurek¹, M. Dawid¹, T. Milewicz², P. Pawlicki³, M. Kotula-Balak⁴, A. Rak¹** (¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Gynecological Endocrinology, Jagiellonian University Medical College, Krakow, Poland, ³Center for Experimental and Innovative Medicine, University of Agriculture in Krakow, Krakow, Poland, ⁴University Centre of Veterinary Medicine JU-UA, University of Agriculture in Krakow, Krakow, Poland).
- S11.P4 PROTEOMIC ANALYSIS OF PORCINE CORPUS LUTEUM DURING THE ESTROUS CYCLE: EFFECTS OF PPAR GAMMA LIGANDS. **Z. Kunicka¹, K. Mierzejewski¹, A. Kurzynska¹, M. Golubska¹, R. Stryński², J. Mateos³, M. Carrera⁴, I. Bogacka¹** (¹Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, ²Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland, ³Galapagos NV, Department of Food Technology, Mechelen, Belgium, ⁴Marine Research Institute (IIM), Spanish National Research Council (CSIC), Vigo, Spain).
- S11.P5 TRANSCRIPTOMIC PROFILE OF OVIDUCTAL ISTHMUS IN PIGS ON DAYS 2 TO 3 OF PREGNANCY. **M. Martyniak, E. Waszkiewicz, A. Franczak, G. Kotwica** (Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Olsztyn, Poland).
- S11.P6 THE EFFECT OF SATURATED FATTY ACIDS ON GnRH-INDUCED GONADOTROPIN SECRETION FROM ANTERIOR PITUITARY CELLS OF PUBESCENT EWE LAMBS. **N. Szysiak, A. Furmanczyk-Gnyp, B. Surowka, B. Szymczak, N. Minakow, U. Kosior-Korzecka** (Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland).
- S11.P7 DIETARY SUPPLEMENTATION WITH NETTLE INDUCES APOPTOSIS AND AFFECTED FOLLICULOGENESIS IN THE RABBIT OVARY **K. Kaminska¹, K. Kapusta¹, S. Palka², M. Kmiecik², J. Zubel-Lojek³, M. Grzesiak¹** (¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Krakow, Krakow, Poland, ³Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Krakow, Poland).
- S11.P8 CHEMERIN IMPACT ON DIFFERENTIALLY EXPRESSED GENES IN THE ENDOMETRIAL TRANSCRIPTOME OF PIGS DURING PERIIMPLANTATION PERIOD. **K. Bors, G. Kopij, L. Paukzsto, M. Kiezun, E. Rytelewska, K. Kisielewska, M. Gudelska, K. Szymanska, K. Dobrzyn, E. Zaobidna, J. Jastrzebski, T. Kaminski, N. Smolinska** (University of Warmia and Mazury, Olsztyn, Poland).
- S11.P9 THE EFFECT OF TYPE 1 DIABETES AND HIGH FAT DIET ON THE EXPRESSION OF RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS (RAGE) IN UTERUS. **K. Zglejc-Waszak¹, A. Korytko¹, J. Wojtkiewicz¹, K. Wasowicz², J.K. Juranek¹** (¹University of Warmia and Mazury in Olsztyn, School of Medicine, Collegium Medicum, Department of Human Physiology and Pathophysiology, Olsztyn, Poland, ²University of Warmia and Mazury in Olsztyn, Faculty of Veterinary Medicine, Department of Pathophysiology, Olsztyn, Poland).
- S11.P10 AQUAPORINS EXPRESSION IN REPRODUCTIVE TRACT OF THE BULL (BOS TAURUS) CHANGES WITH SEXUAL MATURITY. PRELIMINARY STUDY. **P. Oberska¹, P. Malkowska¹, M. Grabowska², M. Murawski³, D. Gaczarzewicz⁴, A. Syczewski⁵, K. Michalek¹** (¹Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology in Szczecin, Szczecin, Poland, ²Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland, ³Department of Animal Nutrition, Biotechnology and

- Fisheries, University of Agriculture in Krakow, Krakow, Poland, ⁴Department of Animal Reproduction, Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Szczecin, Poland, ⁵Genetic and Animal Husbandry, Szczecin, Poland).
- S11.P11 IMPACT OF FETAL NUMBER ON ACUTE PHASE PROTEINS, CORTISOL AND HEMATOLOGICAL PARAMETERS IN EWES DURING THE PERIPARTURIENT PERIOD. **M. Gregula-Kania¹, U. Kosior-Korzecka², K. Kania³, A. Hahaj-Siembida¹** (¹Institute of Animal Breeding and Biodiversity Conservation, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Lublin, Poland, ²Sub-Department of Pathophysiology, Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland, ³Department of BioPhysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Lublin, Poland).
- S11.P12 CHEMERIN EFFECT ON STEROIDOGENESIS IN THE PORCINE UTERUS: AN IN VITRO STUDY. **M. Gudelska, K. Dobrzyn, E. Rytelewska, K. Kisielewska, M. Kiezun, E. Zaobidna, K. Bors, G. Kopij, K. Szymanska, B. Kaminska, T. Kaminski, N. Smolinska** (University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).
- S11.P13 THE IMPACT OF CHEMERIN ON THE SECRETION OF CYTOKINES (IL-1 β , IL-6) AND THE EXPRESSION OF CYTOKINE RECEPTORS (IL1R1, IL6R) BY THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY. **G. Kopij, M. Kiezun, E. Zaobidna, K. Dobrzyn, K. Kisielewska, E. Rytelewska, M. Gudelska, K. Bors, K. Szymanska, B. Kaminska, T. Kaminski, N. Smolinska** (University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).
- S11.P14 ADIPONECTIN AS A PROINFLAMMATORY FACTOR IN THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND IMPLANTATION: AN IN VITRO STUDY. **M. Kiezun, K. Dobrzyn, E. Rytelewska, K. Kisielewska, M. Gudelska, K. Bors, G. Kopij, K. Szymanska, E. Zaobidna, B. Kaminska, T. Kaminski, N. Smolinska** (Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland).
- S11.P15 THE INFLUENCE OF KETOGENIC DIET ON THE COURSE OF GESTATION AND BIOCHEMICAL COMPOSITION OF HIPPOCAMPAL FORMATION OF PREGNANT RATS. **Z. Rauk¹, P. Szulc², W. Kosiek¹, Z. Setkowicz-Janeczko¹** (¹Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, Poland, ²Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, Poland).
- S11.P16 VISFATIN GENE EXPRESSION IN THE PORCINE PITUITARY GLAND DURING THE ESTROUS CYCLE AND EARLY PREGNANCY. **K. Szymanska¹, M. Kiezun¹, E. Zaobidna¹, K. Dobrzyn¹, E. Mlyczynska², E. Rytelewska¹, K. Kisielewska¹, M. Gudelska¹, K. Bors¹, G. Kopij¹, B. Kaminska¹, A. Rak², N. Smolinska¹, T. Kaminski¹** (¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, ²Department of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland).

EPIGENETIC MECHANISMS AND BIO-MECHANICAL CUES DRIVE CELL DIFFERENTIATION

T.A.L. BREVINI¹, G. PENNAROSSA¹, R. PASQUARIELLO¹, F. GANDOLFI¹

¹Laboratory of Biomedical Embryology, Centre for Stem Cell Research, UniStem, Università degli Studi di Milano, Milano, Italy

Multiple regulatory mechanisms interact and orchestrate a proper regulation of gene expression and spatial restriction, to allow cells to adopt distinct differentiation traits and a terminal phenotype. Changes in methylation, for instance, are among the main actors of pluripotency, and under the control of methyltransferases and Ten-eleven Translocation (TET) enzymes, add/or erase phenotype distinct methylation changes along embryo development as well as during mesenchymal to epithelial transition (MET). On the other hand, extracellular factors such as small molecules have the ability to interact with cell plasticity and to induce a transient pluripotent state that allows the direct conversion of an adult mature cell into another differentiated cell type. In addition, mechanical properties of the cellular microenvironment and 3-D rearrangement can affect both cell potency and differentiation, through dramatic effects on cytoskeleton remodelling and with the involvement of specific mechano-sensing-related pathways, such as the Hippo and the RhoGTPase, that are finely able to tune oocyte quality and developmental competence as well as plasticity and differentiation of somatic cells. Here we will discuss the involvement of epigenetic cues and bio-mechanical effector in driving cell potency and differentiation as well as in terminal cell phenotype specification.

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Author for correspondence: Tiziana Brevini (tiziana.brevini@unimi.it)

PRESENCE OF LACTOBACILLI WITHIN THE BOVINE FEMALE REPRODUCTIVE TRACT AND THEIR POSSIBLE ROLE DURING REPRODUCTIVE EVENTS

C. GABLER

Institute of Veterinary Biochemistry, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Uterine diseases in cattle are mainly caused by pathogenic bacteria and these bacterial species have been in the focus of research and treatment over the last decades. However, commensal bacteria were also identified in the bovine reproductive tract of healthy cows by cultivation as well as by sequencing of 16S rRNA with mainly *Lactobacillus* spp. found in vagina and uterus. Significant changes have been observed within the uterine commensal bacterial composition during the first weeks after calving. Higher abundance of *Lactobacillus* spp. in healthy animals indicates an influence on reproductive events. Therefore, such strains have become of particular interest to improving the uterine health status for better fertility rates. There are several characteristics awarded to *Lactobacillus* spp. that explain the potential positive influence on the health of their host. *Lactobacillus* spp. can represent a barrier to infection by suppressing the population of bacterial pathogens through competition for nutrients and production of organic acids, hydrogen peroxide and bacteriocins lethal to pathogens. They can also protect their host from the detrimental effects of pathogens through competition for adherence to epithelial cells and the production of a protective biofilm on the epithelial cell surface. *In vitro* co-culture experiments of *Lactobacillus* spp. with endometrial epithelial cells have demonstrated that several *Lactobacillus* spp. do not affect viability of epithelial cells nor provoke a pro-inflammatory reaction by up to 96 h. Intrauterine applied *Lactobacillus* spp. provoke a weak immune response with a low number of immune cells invading for a short time, as well as an increased mRNA expression of some pro-inflammatory factors after 7 days. Intrauterine administered *L. buchneri* to cows with subclinical endometritis led to improved fertility rates, decreasing the number of days open or insemination attempts. Three weeks after administration, endometrial mRNA expression of several pro-inflammatory factors was lower in the *L. buchneri* group compared to the placebo group. A mixture of *Lactobacillus* spp. were applied intravaginal around the time of calving leading to a decreased number of cases of uterine diseases and/or better fertility rates by decreased days open. The presence of *Lactobacillus* spp. also did not affect sperm viability parameters. In conclusion, the data show that an intact commensal bacterial composition seems to be necessary for improved fertility rates in cattle.

Author for correspondence: Christoph Gabler (christoph.gabler@fu-berlin.de)

THE IMPACT OF ENERGY METABOLISM CHANGE IN PORCINE CUMULUS-OOCYTE COMPLEX DURING *IN VITRO* MATURATION

G. GORCZYCA¹, K. WARTALSKI², M. DUDA¹

¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland,

²Department of Histology, Jagiellonian University Medical College, Krakow, Poland

In the past decade, extensive observations demonstrated that the mitochondria (MT) play a crucial role in the oocyte cytoplasm maturation, since they provide adenosine triphosphate (ATP) for fertilization and preimplantation embryo development. In turn, intracellular lipids, in both oocyte and cumulus cells, are stored mainly in lipid droplets (LD) providing energy for their normal growth and development. Thus, alterations in ovarian lipid profile can impact the cumulus-oocyte complex (COC) during its maturation. The increasing human exposure to agents capable of inducing changes in the genetic material, accumulation of endocrine active compounds (EACs) in the environment might adversely modulate mitochondrial and lipid content. Therefore, the main purpose of this research was to elucidate whether exposure of porcine COCs to selected EACs affects their energy metabolism. The COCs were isolated from healthy, medium-sized porcine follicles (4–6 mm in diameter), encapsulated in alginate beads and then cultured (3D) in the presence of vinclozolin (Vnz; a fungicide), nandrolone (Ndn; an anabolic steroid), and cyclosporin A (CsA; an immunosuppressant). After termination of culture (96 h) COCs were prepared for TEM analysis, Nile Red (NR) staining and Aligent Seahorse XFp Cell Mito Stress Test. The results demonstrated that ATP production in all experimental groups was lower compare to control. Proton leak was highest in COCs cultured with addition of Vnz. Moreover, Vnz induced non-mitochondrial respiration. TEM analysis showed modified distribution and shape of MT in COCs exposed to Ndn and Vnz - changing them from diffuse to aggregate and from spherical to elongated, respectively. It was also observed that LDs in Ndn and Vnz treated COCs were accumulated and solidified. The results of the NR analysis were consistent with the results of the TEM analyses. Concluding, the obtained results indicate a disrupting effect of Ndn and Vnz to COCs energy metabolism, which may adversely affects regulatory mechanisms of maturation and hence in porcine oocyte competence acquisition.

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Author for correspondence: Gabriela Gorczyca (gabriela.gorczyca@doctoral.uj.edu.pl)

SEX STEROID RECEPTOR AGONISTS AND ANTAGONISTS AFFECT THE EXPRESSION OF TRANSCRIPTION FACTOR FORKHEAD L2 (FOXL2) IN THE NEONATAL PORCINE OVARY

P. WITEK, N. MAREK, M. GRZESIAK, M. SLOMCZYNSKA, K. KNAPCZYK-STWORA

Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland

In many mammals the formation of primordial ovarian follicles begins during fetal development and involves the breakdown of egg nests, and subsequent recruitment of pregranulosa cells. In pigs, the formation of the primordial follicle pool is completed around post-partum day 25. Once formed, some primordial follicles are recruited into the primary follicle pool. This process is characterized by differentiation of the flattened pregranulosa cells into cuboidal granulosa cells. Ovarian folliculogenesis is governed by many factors and hormones. Previously, we have demonstrated that neonatal exposure to endocrine active chemicals influenced the number of primordial and primary follicles in piglets. The main factor responsible for the regulation of granulosa cell function is the transcription factor forkhead L2 (FOXL2), which is necessary for the proper formation and activation of ovarian follicles. Thus, the objective of this study was to determine whether exposure of the neonatal pigs to testosterone propionate (TP, an androgen), flutamide (FLU, an antiandrogen), 4-*tert*-octylphenol (OP, compound with estrogenic activity), ICI 182,780 (ICI, an antiestrogen), and methoxychlor (MXC, compound with estrogenic, antiestrogenic and antiandrogenic properties) influenced ovarian *FOXL2* expression as well as the expression of its target genes, *AMH* and *CYP19A1*. Piglets were injected with TP, FLU, OP, ICI, MXC, or corn oil (control) between postnatal days 1 and 10 (n=4/each group). Ovaries were excised from the 11-day-old pigs and to assess *FOXL2*, *AMH*, and *CYP19A1* expression immunohistochemistry and/or real-time PCR and Western blot were performed. *FOXL2* protein was localized in stroma cells surrounding egg nests and in both pregranulosa and granulosa cells. TP, OP, and MXC increased *FOXL2* and *AMH* mRNAs, while FLU and ICI decreased *CYP19A1* mRNA. The *FOXL2* protein abundance was increased in all examined groups. TP, OP, ICI, and MXC increased *AMH* protein abundance, while TP, FLU and OP decreased *CYP19A1* protein abundance. In summary, we showed that the exposure to compounds affecting androgen and estrogen action during the neonatal window of porcine development altered *FOXL2* expression. This may, in part, explain the impaired folliculogenesis in those animals, as we previously described.

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Author for correspondence: Patrycja Witek (patrycja.witek@doctoral.uj.edu.pl)

PRO-APOPTOTIC EFFECT OF VASPIN ON HUMAN PLACENTA BEWO CELLS

M. DAWID, E. MLYCZYNSKA, P. KUROWSKA, M. JUREK, A. RAK

Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research,
Jagiellonian University in Krakow, Krakow, Poland

Vaspin, a member of adipokines was first isolated in 2005 from the visceral adipose tissue of the rat abdominal obesity model (OLETF). It belongs to the serine protease inhibitors and acts through a protein G-coupled receptor - GRP78. The expression of vaspin has been described in many tissues and organs, where it plays a pleiotropic function. Interestingly, recent literature data indicate an anti-apoptotic effect of vaspin in ovarian cells, while its role in placenta has not been explored. The aim of this study was to investigate the effect of vaspin on placental apoptosis by studying Bcl-2, BAX, p53, caspase-8, caspase-9, caspase-3 protein expression in placental BeWo cells. Apoptosis is an essential feature of normal placental development but is exaggerated in association with placental disease. In normal pregnancy, trophoblast apoptosis increases with placental growth and advancing gestation. However, apoptosis is notably exaggerated in the pregnancy complications, hydatidiform mole, pre-eclampsia, and intra-uterine growth restriction (IUGR). Human placenta choriocarcinoma cell line BeWo (ATCC®CCL-98™) were cultured in DMEM/F12 medium with 1% FBS and vaspin at doses: 0.1, 1, 10 ng/ml for 24, 48, 72 h of apoptotic factors: Bcl-2, BAX, p53, caspase-8, caspase-9 and caspase-3 by Western blot. Statistical analysis were performed using GraphPad Prism 5 and a one-way ANOVA test ($p < 0.05$). We examined that vaspin at various doses and after different incubation times increased the expression of pro-apoptotic proteins such as: BAX, p53, caspase-8, caspase-9, caspase-3. On the other hand, with regard to the expression of the anti-apoptotic protein Bcl2, it demonstrate the opposite effect. In conclusion, our preliminary studies indicated pro-apoptotic effect of vaspin on BeWo cells. Presented data suggest that adipokine may be an important regulator of the apoptosis process, but also play crucial role as a marker and initiator of numerous pregnancy pathologies, such as preeclampsia or IUGR.

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Author for correspondence: Monika Dawid (monika.dawid@student.uj.edu.pl)

RELATIVE ABUNDANCE OF APELIN RECEPTOR TRANSCRIPT IN THE PORCINE PITUITARY DURING THE ESTROUS CYCLE AND EARLY PREGNANCY

K. KISIELEWSKA, E. RYTELEWSKA, M. GUDELSKA, M. KIEZUN, K. DOBRZYN, E. ZAObIDNA, K. BORS, G. KOPIJ,
K. SZYMANSKA, B. KAMINSKA, N. SMOLINSKA, T. KAMINSKI

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

The current knowledge on role of the adipose tissue suggests that it is also an endocrine organ. It secretes biologically active compounds, named adipokines. Previous research demonstrated an involvement of some adipokines in the proper functioning of the female reproductive system in pigs by acting at all branches of the hypothalamic-pituitary-ovarian axis. Apelin also seems to be a hormone involved in the regulation of both, the reproduction and energy homeostasis. The aim of this study was to investigate apelin receptor (*APLNR*) mRNA expression in the porcine anterior (AP) and posterior (PP) pituitary on days 2–3, 10–12, 14–16 and 17–19 of the estrous cycle, as well as on days 10–11, 12–13, 15–16 and 27–28 of gestation. Expression of *APLNR* mRNA was evaluated using real-time PCR method. Data were analysed using one-way ANOVA. In the AP, during the estrous cycle, the relative abundance of *APLNR* mRNA transcript was higher on days 2–3, when compared to days 14–16. During early pregnancy, the higher mRNA content was observed on days 15–16, whereas lower on days 27–28. Comparing early gestation stages and days 10–12 of the estrous cycle, there were no significant changes in *APLNR* mRNA content between the cycle and early pregnancy. In the case of PP, relative abundance of *APLNR* mRNA was higher on days 10–12 and 17–19, when compared to days 2–3 of the estrous cycle. During early pregnancy period, the highest relative abundance of *APLNR* transcript was observed on days 27–28. Comparison of the cycle and early pregnancy revealed increased *APLNR* mRNA level on days 27–28 of pregnancy, when compared to days 10–12 of the cycle. Our studies demonstrated the presence of *APLNR* mRNA in the both porcine pituitary lobes. Moreover, the provided data showed that the level of *APLNR* transcript fluctuates during the estrous cycle and early pregnancy, which suggests that porcine pituitary may show various sensitivity to the apelin action throughout the estrous cycle and early pregnancy.

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Author for correspondence: Katarzyna Kisielewska (katarzyna.kisielewska@uwm.edu.pl)

VISFATIN/NAMPT EXPRESSION IN PORCINE CORPUS LUTEUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY. EFFECT OF LUTEINIZING HORMONE AND PROGESTERONE ON VISFATIN PROTEIN LEVEL

E. MLYCZYNSKA¹, E. ZAOBIDNA², E. RYTELEWSKA², M. KIEZUN², K. DOBRZYN², N. SMOLINSKA², T. KAMINSKI², A. RAK¹

¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland, ²Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn-Kortowo, Poland

Visfatin, also termed nicotinamide phosphoribosyltransferase (NAMPT), is the adipose tissue derived hormone with a mimetic effect to insulin, responsible for maintaining energy homeostasis, regulation of angiogenesis and inflammation. Last studies documented visfatin effect on female reproduction both at central and gonadal level; its expression was noted in the human, mouse, chicken, bovine, and hen ovarian follicular cells. Moreover, it was shown that visfatin enhanced basal and IGF1-induced steroid hormone secretion by the bovine granulosa cells, however the data on its role in the porcine reproduction are still limited. The aim of the study was to determine the expression of visfatin in the porcine corpus luteum (CL) during the oestrous cycle and early pregnancy, and then examine the direct effect of luteinizing hormone (LH) and progesterone (P₄) on visfatin protein expression in luteal cells. Luteal tissue samples were harvested from gilts on days 2–3, 10–12, and 14–16 of the oestrous cycle, and days 10–11, 12–13, 15–16, 27–28 of pregnancy (n=6 per group). The expression of the visfatin gene (NAMPT) was measured by quantitative real time PCR, while the protein level by Western blot. Next, the ovaries of the gilts (n=5 per group) on days 2–3, 10–12 and 14–16 of the oestrous cycle were used to determine the impact of hormones: LH (100 ng/ml) and P₄ (10, 100, 1000 nM) on visfatin protein abundance. Differences between groups were analysed by one-way ANOVA followed by Tukey's *post hoc* test. We observed the highest expression of NAMPT in CL collected on days 2–3 and 14–16 of the oestrous cycle, and days 12–13 to 15–16 of pregnancy. Conversely, at the protein level, the greatest expression of visfatin was found on days 10–12 of the oestrous cycle and on days 15–16 to 27–28 of pregnancy. Additionally, we noticed that both LH and P₄ increased significantly visfatin protein abundance: LH on days 2–3 and 14–16, P₄ on days 2–3 and 10–12 of the oestrous cycle. Our results indicates that visfatin expression in luteal cells changed during the oestrous cycle and early pregnancy; moreover both LH and P₄ increased luteal expression of visfatin. In conclusion, our preliminary study indicates that visfatin is the adipokine which can be a new potential regulator of CL function in pigs.

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CHEMERIN AS A HORMONE MODULATING ENDOMETRIAL REMODELING IN PIGS DURING THE PERI-IMPLANTATION PERIOD: AN *IN VITRO* STUDY

E. RYTELEWSKA, M. KIEZUN, K. DOBRZYN, E. ZAOBIDNA, M. GUDELSKA, K. KISIELEWSKA, K. BORS, G. KOPIJ, K. SZYMANSKA, B. KAMINSKA, T. KAMINSKI, N. SMOLINSKA

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

The implantation in pigs is superficial and non-invasive, including phases of apposition, adhesion and attachment of conceptuses to the endometrial surface. This process is related to the remodeling of connective tissue and the extracellular matrix. Matrix metalloproteinases (MMPs) are the proteolytic enzymes that degrade the extracellular matrix and are essential for the tissue remodeling processes. In turn, the tissue inhibitors of metalloproteinases (TIMPs) are important regulators of MMPs activity. According to our recent studies, chemerin - a hormone which participates in the regulation of energy homeostasis and the immune response - may also be involved in the regulation of porcine uterus functioning. Hence, the aim of this study was to investigate the *in vitro* effect of chemerin, at the physiological concentrations, on the protein abundance of crucial metalloproteinases (matrix metalloproteinase 2 (MMP-2); matrix metalloproteinase 9 (MMP-9)) and tissue inhibitors of metalloproteinases (tissue inhibitor of metalloproteinases 1 (TIMP-1); tissue inhibitor of metalloproteinases 2, (TIMP-2)) in the porcine endometrium during the peri-implantation period. Endometrial tissue explants (n=5 per period) were harvested from sows during the beginning of implantation (days 15 to 16 of pregnancy) and the end of implantation (days 27 to 28 of pregnancy). Tissue explants were preincubated for 2 h and then incubated for 24 h with chemerin (at the doses of 100 and 200 ng/mL) or medium without any treatment (controls). The protein abundance of the target proteins was determined by Western Blot. The results were analyzed by one-way analysis of variance followed by Duncan's *post hoc* test. The study revealed that chemerin (at both studied doses) enhanced the protein abundance of TIMP-1 and TIMP-2, and decreased the abundance of MMP-2 and MMP-9 during the beginning of implantation. In turn, the opposite results were obtained during the end of implantation, when chemerin (100 and 200 ng/mL) enhanced the protein abundance of MMPs, and decreased the abundance of TIMPs. Therefore, the obtained results confirm that chemerin, at physiological concentrations, may affect endometrial remodeling in pigs during the peri-implantation period.

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DECORIN AND DERMATOPONTIN DIFFERENTIALLY AFFECT CARUNCULAR EPITHELIAL CELL ADHESION IN PREGNANT COWS

M. JAMIOL, J. WAWRZYKOWSKI, M. KANKOFER

Department of Biochemistry, Faculty of Veterinary Medicine, University of Life Science in Lublin, Lublin, Poland

Proper placental development and maturation depend on cell-cell and cell-ECM interactions mediated by ECM proteins, which include glycoproteins and proteoglycans. Biological properties of ECM proteins, whose profiles dynamically change during pregnancy, allow them to play a key role in the processes of cell adhesion and invasion into the endometrium during placentation and separation of fetal membranes postpartum. Among them decorin (DCN) and dermatopontin (DPT) seem to play a significant role in the functioning of the placenta. They interact with each other and their presence was confirmed in placental tissues of cows during pregnancy. Both DCN and DPT are small protein molecules considered to have a role in the formation of the extracellular matrix and cell adhesion. The aim of the study was to evaluate the effect of DCN and DPT on the adhesion of caruncular epithelial cells derived from cows during early-mid pregnancy. Caruncular epithelial cells were isolated from pregnant cows (2nd, n=2; 4th month, n=2) and used for the examination of the influence of DCN 10 (µg/mL) and DPT (5, 50 and 100 ng/mL) on cell adhesion. The adhesion of cells to fibronectin was measured spectrophotometrically. The MTT assay was used to evaluate the effect of selected proteins on the viability of placental cells. DCN limited the adhesion of cells in the 2nd month of pregnancy, whereas DPT was shown to have pro-adhesive activity both in the 2nd and 4th month of pregnancy. The results obtained here indicate that both proteins, showing the opposite effect, may influence cell adhesion during attachment and most probably also detachment of bovine placenta. Further studies on mechanisms of action of DPT and DCN in bovine placenta are necessary.

Author for correspondence: Monika Jamiol (monika.jamiol@up.lublin.pl)

THE ELECTROMAGNETIC FIELD (EMF) RADIATION INDUCES TRANSCRIPTOMIC ALTERATIONS IN PIG MYOMETRIUM DURING THE PERI-IMPLANTATION PERIOD

E.M. Drzewiecka¹, W. Kozłowska¹, L. Paukszto², A. Zmijewska¹, P.J. Wydorski¹, J.P. Jastrzebski², A. Franczak¹

¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, ²Department of Plant Physiology, Genetics and Biotechnology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

There are documented effects of the electromagnetic field (EMF) radiation on physiological processes in mammals including gametes production and regulation of reproductive cycle, the function of the uterus, and pregnancy outcome. However, the molecular background of the EMF-induced alterations remains not sufficiently established. In this study, the myometrial slices obtained from pigs during the peri-implantation period (days 15–16 of pregnancy, n=5) were exposed to an EMF at a frequency of 50 Hz within a short-term duration (2 h) for further transcriptomic profiling using a next-generation sequencing (NGS) method followed by validation procedure using real-time PCR. As result, the EMF radiation affected the expression of 215 transcript active regions (TARs), and among them, the assigned gene name possessed 90 ones (differentially expressed genes, DEGs). Among the evaluated DEGs there were genes encoding interleukin-15 (IL15), tumor necrosis factor (TNF), prodynorphin (PDYN), homeobox-D13 (HOXD13), signal transducer and activator of transcription-5A (STAT5A), vascular cell adhesion molecule-1 (VCAM1), and early growth response protein-2 (EGR2). The evaluated DEGs are categorized mostly to gene ontology biological processes terms connected with defense and immune responses, and secretion and export. Mostly enriched KEGG pathways were TNF signaling pathway, and regulation of IFNA signaling, and there was found REACTOME interferon alpha/beta signaling pathway. In conclusion, the EMF within a short duration of treatment mostly affects genes involved in defense and immune responses to different factors in the myometrium, which can affect the proper course of molecular events accompanying the peri-implantation period.

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Author for correspondence: Anita Franczak (anitaf@uwm.edu.pl)

PPAR-GAMMA REGULATES THE EXPRESSION OF GENES IN THE PORCINE INFLAMED ENDOMETRIUM DURING FOLLICULAR PHASE OF THE ESTROUS CYCLE

K. MIERZEJEWSKI¹, L. PAUKSZTO², A. KURZYNSKA¹, Z. KUNICKA¹, J.P. JASTRZEBSKI², M. GOLUBSKA¹, I. BOGACKA¹

¹University of Warmia and Mazury in Olsztyn, Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology, Olsztyn, Poland,

²University of Warmia and Mazury in Olsztyn, Faculty of Biology and Biotechnology, Department of Plant Physiology, Genetics and Biotechnology, Olsztyn, Poland

Peroxisome proliferator-activated receptors (PPARs) belong to a ligand-dependent nuclear receptor family. Numerous studies have revealed the presence and significance of different PPAR isoforms in the reproductive system. In this study, we determine the effect of PPAR gamma ligands - agonists: 15d-prostaglandin J2 (PGJ₂) or pioglitazone (P) and antagonist: T0070907 (T) on a global transcriptome profile in the porcine LPS-stimulated endometrium, incubated *in vitro*. To identify expression profiles of endometrial genes the RNA-Seq was performed on the NovaSeq 6000 Illumina platform. To identify differentially expressed genes (DEGs) we applied 74 Cufflinks method. The final results constituted DEGs, significantly confirmed by statistical test (adjusted p-value <0.05). Enrichment gene ontology and pathway analysis were performed with use of gProfileR based on Gene Ontology (GO), and Kyoto Encyclopaedia of Genes and Genomes (KEGG) databases. Within all experimental comparisons we detected 187, 476, 557 and 230 DEGs for PGJ₂ vs. LPS, P vs. LPS, T vs. LPS and LPS vs. control, respectively. The results revealed the engagement of PPAR γ ligands in various immunological processes, including IL-1 β production, IL-17 signalling pathway, defence response. Most of the described DEGs have been assigned to p53 signalling pathway. Moreover, the study provide relevant finding related to the ability of pioglitazone to regulate the expression of genes (GADD45 β , CDK1, CCNG1, CCNA1) controlling DNA damage repair upon stress-triggered conditions. These numerous results provide a basis for further studies on PPAR γ mechanisms controlling reproductive functions during inflammation.

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Author for correspondence: Karol Mierzejewski (karol.mierzejewski@uwm.edu.pl)

KISS-1/GPR54 MRNA EXPRESSION AND THE RELATIONSHIP BETWEEN KISS-10 AND LUTEINIZING HORMONE SECRETION IN PITUITARY GLAND OF CYCLIC AND POLYCYSTIC OVARY SYNDROME-AFFECTED SOWS

U. KOSIOR-KORZECKA¹, V. LONGO², C. DELLA CROCE², N. SZYSIAK¹, A. FURMANCZYK-GNYP¹, A. NOWAKIEWICZ¹, I. PUZIO³, B. SUROWKA¹, N. MINAKOW¹, B. SZYMCZAK¹

¹Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland,

²National Research Council, Institute of Agricultural Biology and Biotechnology, Pisa, Italy,

³Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland

The aim of study was to compare the effect of kisspeptin-10 (KiSS-10) on *in vitro* luteinizing hormone (LH) secretion by pituitary cells of cyclic sows (n=10) and sows with follicular cysts (n=12). In addition, the concentration of KiSS-10 in the blood plasma and pituitary *kiss-1/gpr54* mRNA expression in both groups of animals were determined. Pituitary cells were cultured in McCoy 5A medium without hormones (the negative control), with GnRH (4×10^{-9} M; the positive control), with KISS-10 (10^{-11} – 10^{-7} M) or with both GnRH (4×10^{-9} M) and KISS-10 (10^{-11} – 10^{-7} M). After 2, 6, 12, 18, 24 and 30 hours of the experiment, the media for LH analysis were collected and the proliferation index (PI) of the control cells and those treated with KISS-10 (10^{-11} – 10^{-7} M) or both GnRH (4×10^{-9} M) and KiSS-10 (10^{-11} – 10^{-7} M) was determined. KiSS-10 in the blood plasma and LH in the culture medium were determined by ELISA assays using species-specific antibodies. The obtained results show that plasma KiSS-10 concentration was higher in cyclic sows compared to those polycystic ovary syndrome (PCOS)-affected. Pituitary *kiss-1 mRNA* expression was lower whereas *gpr54* mRNA expression was higher in sows with follicular cysts than in cyclic sows. The *in vitro* effect of KiSS-10 on pituitary cells isolated from cyclic sows and sows with follicular cysts depended on the KiSS-10 concentration used. In the 10^{-9} – 10^{-7} M concentration, KiSS-10 exerted a stimulatory effect on LH secretion *in vitro* in both groups, with the highest LH secretion observed under the influence of 10^{-8} M of KiSS-10. Despite of high positive correlation between the concentration of KiSS-10 and LH secretion both in cultures without GnRH and with GnRH, the level of KiSS-10-stimulated LH secretion was significantly lower in cells isolated from PCOS-affected sows than from cyclic sows.

Author for correspondence: Urszula Kosior-Korzecka (urszula.korzecka@up.lublin.pl)

KISS-INDUCED ALTERNATIONS IN *PRL* MRNA TRANSCRIPT ABUNDANCE IN PORCINE PITUITARY CELLS DURING THE ESTROUS CYCLE

A. ZMIJEWSKA¹, W. CZELEJEWSKA^{1,2}, E.M. DRZEWIECKA¹, S. OKRASA¹, A. FRANCAK¹

¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,

²Department of Neurosurgery Laboratory of Regenerative Medicine, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Kisspeptins (KISS) affect the synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and act as the principal positive regulators of female reproductive axis. The question arises if KISS may also contribute to the regulation of prolactin (PRL) synthesis in the pituitary gland. The study aims to determine the *in vitro* effect of KISS on *PRL* mRNA transcript abundance in pituitary glands collected from pigs on days 2–3, 10–12, 15–16, and 19–20 of the estrous cycle. The pituitary cells were cultured *in vitro* and treated with KISS (10^{-6} M, 10^{-7} M) for four hours. The abundance of the *PRL* mRNA transcript was examined by real-time PCR. The expression of the *PRL* mRNA was elevated by the KISS at doses 10^{-6} M and 10^{-7} M in porcine pituitary cells collected during the early-luteal (days 2–3) and mid-luteal (days 10–12) phase of the estrous cycle. Moreover, the stimulatory effect of the KISS at a dose 10^{-6} M on *PRL* mRNA transcript abundance was observed during luteolysis and follicular phase of the estrous cycle. In conclusion, KISS may be recognized as a modulator of PRL synthesis in porcine pituitary cells during the estrous cycle.

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Author for correspondence: Agata Zmijewska (agata.zmijewska@uwm.edu.pl)

EFFECTS OF LONG-TERM CHANGES IN BODY WEIGHT ON THE ABILITY OF RESISTIN TO MODULATE REPRODUCTIVE HORMONES IN SHEEP

M. SZCZESNA, W. BIERNAT, K. KIRSZ, D.A. ZIEBA

Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Sciences, University of Agriculture in Krakow, Krakow, Poland

Adipokines are hormones that are mainly produced by white adipose tissue, an endocrine organ involved in energy homeostasis. They play important roles in the regulation of lipid and glucose metabolism, inflammation and immune disorders. New roles for adipokines have recently emerged in the field of fertility and reproduction, particularly since leptin was described. Indeed, the adipokine resistin (RSTN) is able to regulate the functions of male and female gonads and of the hypothalamic-pituitary axis in primates. Fertility is strongly dependent on metabolic status of the organism; thus, matching reproductive activity to nutritional reserves is fundamental to the survival of a species. Both, long-term undernutrition and overnutrition influence reproductive potential and this linkage is mediated in part through the action of two important adipokines, leptin and resistin. In the current study, we manipulated the diet of ewes over 4 months to produce either a thin (Lean) or fat (Fat) body condition and investigated how resistin affects reproductive and metabolic status under low (thin sheep) or high (fat sheep) circulating levels of leptin. Twenty ovariectomized ewes with estrogen replacement were assigned to one of four groups (n=5 per group): Lean-S (n=5) and Fat-S (n=5) groups which were treated with saline and Lean-R (n=5) and Fat-R (n=5) groups treated intravenously one time with recombinant bovine resistin (rbresistin; 5.0 µg/kg BW). Jugular blood samples (5 mL) were collected at 10-min intervals over 4 h *via* indwelling catheters to establish reproductive hormone status before and after resistin challenge. Plasma was assayed for LH, FSH, PRL. Resistin decreased (P < 0.001) plasma LH in both Lean and Fat groups relative to saline controls, with effects on both amplitude and frequency of pulses. Varying effects of resistin were also observed on both plasma FSH and prolactin and were dependent upon nutritional status. Results demonstrate that resistin is intimately involved in the regulation of reproductive hormone secretion in the female and these effects are modulated by extremes in metabolic status (lean vs fat) that can contribute to ovarian pathophysiology. RSTN appears to be another adipokine, in addition to leptin, that is involved in the regulation of reproductive processes in sheep. RSTN regulates the release of reproductive hormones from the pituitary. The study needs further investigation since resistin is able to modulate LH pulse characteristics, it can be speculated that RSTN can work on hypothalamus level in sheep.

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Author for correspondence: (rzzieba@cyf-kr.edu.pl)

VASPIN (VISCERAL ADIPOSE TISSUE-DERIVED SERINE PROTEASE INHIBITOR) ENHANCED PORCINE OOCYTES *IN VITRO* MATURATION *VIA* MAP3/1 AND PRKAA1 KINASES PATHWAYS

P. KUROWSKA¹, M. MLYCZYNSKA¹, A. ESTIENNE², A. BARBE², I. RAJSKA³, K. SOBOL³,
K. PONIEDZIALEK-KEMPNY³, J. DUPONT², A. RAK¹

¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland, ²INRAE, UMR85, Unite Physiologie de la Reproduction et des Comportements, Nouzilly, France, ³Department of Reproductive Biotechnology and Cryopreservation, National Research Institute of Animal Production, Balice, Poland

Oocyte maturation is a critical stage of embryo production in mammals. Adipokines, hormones produced by adipose tissues, are important regulators of whole-body physiology including reproduction. Our previous research showed that adipokine vaspin regulates porcine ovarian follicle's function e.g., stimulates steroidogenesis, proliferation and inhibits apoptosis. Hence, in the present study, we measured porcine cumulus-oocyte complexes (COCs) mRNA and protein expression of vaspin and its receptor 78-kDa glucose-regulated (GRP78) before and after oocytes *in vitro* maturation (IVM) by real-time PCR and Western blot. Moreover, we investigated vaspin/GRP78 localization in COCs by immunofluorescence and the effects of vaspin on oocyte IVM, as well as the molecular mechanism of its action. Porcine COCs were matured *in vitro* for 22 h or 44 h with vaspin (1 ng/mL) and then nuclear maturation assessed by Hoechst 33342 or DAPI staining and additionally by the measurement of progesterone (P4) level in the maturation medium and mitogen activated kinase (MAP3/1), as well as AMP activated kinase (PRKAA1) phosphorylation. As first, we demonstrated that vaspin and GRP78 protein expression increased in oocytes and cumulus cells after IVM. Furthermore, vaspin stimulated porcine oocyte IVM and P4 concentration, as well as MAP3/1 phosphorylation, with opposite effects on PRKAA1. Molecular mechanism was studied using pharmacological inhibitors of MAP3/1 (PD98059) and PRKAA1 (compound C); we investigated, that the effect of vaspin was reversed to the control level in all studied parameters. To conclude, vaspin, by improving *in vitro* oocyte maturation *via* MAP3/1 and PRKAA1 kinase pathways, can be a new factor to accomplish *in vitro* fertilization protocols and production of farm animals.

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Author for correspondence: Patrycja Kurowska (patrycja.kurowska@doctoral.uj.edu.pl)

ELECTROMAGNETIC FIELD OF EXTREMELY LOW FREQUENCY INDUCES CHANGES IN THE RELATIVE ABUNDANCE OF *HSD17B2* AND *VDR* IN THE ENDOMETRIUM OF PIGS DURING THE PERI-IMPLANTATION PERIOD

W. KOZLOWSKA, E.M. DRZEWIECKA, A. ZMIJEWSKA, P.J. WYDORSKI, A. FRAN CZAK

Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

The extremely low-frequency electromagnetic field (EMF) originating from human-made sources becomes a part of the environment that may influence female reproduction. Our past studies indicate that the EMF affects the synthesis of androgens and estrogens in the uterus during the peri-implantation period. The goal of this study was to determine if the EMF (50 Hz, 2 h treatment duration) induces changes in the expression of hydroxysteroid 17 β dehydrogenase 2 (*HSD17B2*) that metabolizes potent estradiol-17 β (E₂) to weakly estrogenic estrone (E₁). It was also documented that vitamin D acting *via* vitamin D receptor (*VDR*) affects the synthesis of estrogen in the uterus. For this reason, this study aimed also to determine the effect of the EMF radiation on *VDR* mRNA transcript abundance in endometrial slices of pigs during the peri-implantation period (n=5). The next-generation sequencing (NGS) provided evidences that the expression of endometrial *HSD17B2* and *VDR* mRNA transcripts alter in the response to EMF radiation. To confirm the NGS results, the *HSD17B2* and *VDR* mRNA transcript abundances were determined with one-step real-time PCR with TaqMan probes. The results of this study confirmed that endometrial slices exposed *in vitro* to the short-term duration of EMF treatment express an increased abundance of *VDR* and a decreased *HSD17B2* mRNA transcript abundance. The observed alterations in *HSD17B2* and *VDR* expression in the endometrium may lead to hyper-concentration of estrogens in the intra-uterine environment and induce cytotoxic effects on embryos during the peri-implantation period. These results could become a basis for further studies explaining the consequences of EMF treatment in the reproductive system.

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Author for correspondence: Wiktoria Kozłowska (wiktoria.kozłowska@uwm.edu.pl)

EFFECT OF VITAMIN D₃ ADMINISTRATION ON GLUCOSE AND INSULIN LEVEL, AND HOMA-IR INDEX IN RATS WITH LETROZOLE-INDUCED POLYCYSTIC OVARY SYNDROME

M. GRZESIAK¹, K. KAMINSKA¹, O. FRACZEK¹, A. SZLAGA², A. MASLANKA³, D. KLIMCZYK³, P. SAMBAK², P. DZIUIROWICZ¹, K. KNAPCZYK-STWORA¹, A. BLASIAK², A. RAK³

¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ³Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age. It is characterized by hyperandrogenism, anovulation, ovarian cysts, and metabolic disorders, such as insulin resistance and hyperinsulinemia. Decreased vitamin D₃ (VD) level was also found among PCOS patients and beneficial effects of VD in PCOS treatment were reported. The aim of the present study was to examine the effect of VD administration on plasma glucose and insulin concentration, and homeostatic model assessment for insulin resistance (HOMA-IR) in PCOS-induced rats. The research was conducted on rats divided into four experimental groups (n=8 per each group): 1) control (C); 2) supplemented with VD (VD; 500IU daily); 3) treated with letrozole (L; 1 mg/kg body weight/daily) and 4) treated with letrozole and VD together (L+VD). After blood collection, total plasma VD, fasting glucose (FG) and insulin (FINS) concentrations were assessed and HOMA-IR was calculated as follow: $FG \text{ (mmol/l)} \times FINS \text{ (}\mu\text{U/ml)} / 22.5$. VD level was significantly higher in VD-supplemented group, while lower in the group with induced PCOS, when compared to the C group. In the L+VD group, the VD concentration markedly increased in comparison to the L group. Glucose and insulin concentrations were the highest in rats with PCOS, whereas VD supplementation (L+VD group) showed the tendency to decreased them. Similarly, HOMA-IR was significantly greater in the L group when compared to the C group with tendency to decreasing in the L+VD group. Our results obtained on rat PCOS model indicate that VD supplementation has a promising potential to improve metabolic parameters in PCOS, including insulin sensitivity. However, further studies on adequate VD dose are required.

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Author for correspondence: M. Grzesiak (m.e.grzesiak@uj.edu.pl)

PROTEIN EXPRESSION AND IMMUNOLocalISATION OF VASPIN AND GRP78 RECEPTOR IN HUMAN PLACENTA OF INTRAUTERINE GROWTH RESTRICTION. PRELIMINARY DATA

M. JUREK¹, M. DAWID¹, T. MILEWICZ², P. PAWLICKI³, M. KOTULA-BALAK⁴, A. RAK¹

¹Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Gynecological Endocrinology, Jagiellonian University Medical College, Krakow, Poland, ³Center for Experimental and Innovative Medicine, University of Agriculture in Krakow, Krakow, Poland, ⁴University Centre of Veterinary Medicine JU-UA, University of Agriculture in Krakow, Krakow, Poland

Intrauterine growth restriction (IUGR) is a serious pathological complication associated with compromised fetal development during pregnancy. IUGR is often linked with impaired placental development, structure and morphology, which in turn alter placental function and capacity of delivering nutrients to the fetus. Various factors including adipokines influence transfer of substances between maternal and fetal circulations. Vaspin (visceral adipose tissue-derived serine protease inhibitor) is known regulator of energy balance, it decreases food intake, promotes preadipocytes differentiation, improves insulin sensitivity and glucose tolerance, and plays an important role in female reproduction. The aim of the present study was to investigate protein expression and immunolocalisation of vaspin and its receptor GRP78 in the maternal and fetal parts of the human placenta of healthy pregnant women (control) and with IUGR. Placental tissue was collected in a gynaecological hospital in Krakow, Department of Gynecological Endocrinology, Jagiellonian University Medical College, Poland, where the clinical information on pregnancy outcomes was obtained. Western Blot and immunohistochemistry methods were used to analyze vaspin and GRP78 expression. Statistical analysis were carried out in GraphPad Prism 5 and a one-way ANOVA test ($p > 0.05$). We observed that protein expression of both vaspin and GRP78 significantly decreased in the fetal part of placenta with IUGR compared to control. Immunohistochemical localization of vaspin revealed its presence in both control and IUGR placenta. In control, positive signal of strong intensity was present in maternal plate, syncytiotrofoblast and fetal plate. In IUGR, the immunosignal was the weakest in fetal plate. Immunosignal for GRP78 was revealed in both control and IUGR placenta, however in control, GRP78 showed moderate signal intensity in all placenta parts when compared to IUGR placenta. In conclusion, our study for the first time showed the immunoexpression and immunolocalisation of vaspin and GRP78 in the maternal and fetal parts of placentas from healthy and complicated by IUGR pregnancies, indicating vaspin as a new regulator in placenta cells. Interesting, protein expression of vaspin/GRP78 was significantly lower in the placenta of IUGR. Future studies will be necessary for understanding the role of vaspin on placenta physiology providing new insights into the pathology of IUGR.

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Author for correspondence: Malgorzata Jurek (malgorzata99.jurek@student.uj.edu.pl)

PROTEOMIC ANALYSIS OF PORCINE CORPUS LUTEUM DURING THE ESTROUS CYCLE: EFFECTS OF PPAR-GAMMA LIGANDS

Z. KUNICKA¹, K. MIERZEJEWSKI¹, A. KURZYNSKA¹, M. GOLUBSKA¹, R. STRYINSKI²,
J. MATEOS³, M. CARRERA⁴, I. BOGACKA¹

¹Faculty of Biology and Biotechnology, Department of Animal Anatomy and Physiology,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland, ²Department of Biochemistry,
Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,
³Galapagos NV, Mechelen, Belgium, ⁴Marine Research Institute (IIM), Spanish National Research Council (CSIC), Vigo, Spain

The corpus luteum (CL) is an endocrine gland present in the ovary of mature females during the estrous cycle as well as pregnancy. There is evidence indicating the relationship between secretory function of the CL and peroxisome proliferator-activated receptors (PPARs). In this study, we investigate the impact of PPAR γ ligands on the proteomic profile of the CL during the mid-luteal phase (days 10–12) and late-luteal phase (days 14–16) of the estrous cycle. The CL slices were incubated *in vitro* for 6 h in the presence of PPAR γ ligands (agonist pioglitazone, antagonist T0070907) or without ligands (control; n=4 for each group). Global proteomic analysis was performed by TMT-based LC MS/MS method. We identified in total 586 proteins in the pig's corpus luteum. Comparative proteomics analysis indicated that 7 various proteins were differentially regulated (DRPs, significantly confirmed by Kruskal-Wallis one-way analysis of variance, adjusted p-value <0.05) in the CL tissue treated with PPAR ligands. In the mid-luteal phase one protein, CAND1, was downregulated after T0070907 treatment. In the group of the upregulated DRPs in the late-luteal phase of the CL treated with pioglitazone we identified: SPTAN1, GOLGB1, TP53BP1, MATR3, RRBP1 and SRRT. Three of them - SPTAN1, GOLGB1 and TP53BP1, were also upregulated in the CL in the late-luteal phase treated with T0070907. Interestingly, CAND1 and RRBP1 are a potential prognostic biomarkers in various types of cancers, due to their involvement in tumor formation and progression. Moreover, the mid-luteal phase control vs. late-luteal phase control comparison analysis showed that certain proteins constitute a specific proteomic signature for each of the examined phases, i.e., 23 and 28 proteins for the mid- and late-luteal phase, respectively. These results provide a basis for further research on the influence of PPAR γ ligands on the expression of tumor biomarkers.

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Author for correspondence: Zuzanna Kunicka (zuzanna.kunicka@uwm.edu.pl)

TRANSCRIPTOMIC PROFILE OF OVIDUCTAL ISTHMUS IN PIGS ON DAYS 2 TO 3 OF PREGNANCY

M. MARTYNIAK, E. WASZKIEWICZ, A. FRAN CZAK, G. KOTWICA

Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

The proper activity of oviducts is crucial for fertilization, embryo development and its transfer to the uterus. It was hypothesized that the presence of embryos in the pig oviducts may evoke alterations in transcriptomic profile of oviductal isthmus. The transcriptomic profile of oviductal isthmus was determined in pigs on days 2–3 of pregnancy and compared with the transcriptome of the tissue during days 2–3 of the estrous cycle. The porcine (V2) expression microarrays 8×60K were used. The analysis indicated 1.91% differentially expressed genes (DEGs) ($P \leq 0.05$; Fold-change ≥ 2.0) and 32.78% of them were up-regulated. Up-regulated DEGs were grouped to gene ontologies (GO) and categorized into functional pathways. Analysis of the relationships among DEGs in isthmus on days 2–3 of pregnancy showed that altered genes are mainly involved in the regulation of endocrine and immune functions, signal transduction and molecule interaction. The largest amount of DEGs was involved in environmental information processing. The presence of embryos in the oviduct induces alterations in transcriptomic profile of pigs oviductal isthmus.

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Author for correspondence: Marcin Martyniak (marcin.martyniak@uwm.edu.pl)

THE EFFECT OF SATURATED FATTY ACIDS ON GNRH-INDUCED GONADOTROPIN SECRETION FROM ANTERIOR PITUITARY CELLS OF PUBESCENT EWE LAMBS

N. SZYSIAK, A. FURMANCZYK-GNYP, B. SUROWKA, B. SZYMCZAK, N. MINAKOW, U. KOSIOR-KORZECKA

Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine,
University of Life Sciences in Lublin, Lublin, Poland

Recently we have shown that the pathologically changed pattern of gonadotropin secretion, responsible for ovulation disorders in fatty ewes, results from the prolonged increase in leptin concentration as well as from diminution of leptin receptors (OB-Rb and OB-Ra) mRNA expression in anterior pituitary cells. Leptin acting peripherally reduces the secretion of insulin - the potent inhibitor of lipolysis. Consequently, the increment in plasma fatty acids level is observed. We also found that in ewe lambs born to obese sheep carrying twins or triplets, high plasma level of saturated fatty acids (SFA) was in positive correlation with the delay in puberty. The relationship between SFA and gonadotropin secretion from the ovine pituitary cells in pubescent ewe lambs is not clear, we resolved to study the effect of SFA on LH and FSH secretion stimulated with GnRH. Moreover, leptin action on gonadotropin secretion in ewes is mediated by nitric oxide. In our study we analysed SFA effect on NO release and also the correlation between gonadotropins and NO under the influence of saturated fatty acid. Pituitary glands were isolated from 7 months old ewe lambs. Pituitary cells were cultured in McCoy 5A medium without GnRH and SFA (negative control), with GnRH only (positive control), with GnRH and 10^{-9} – 10^{-3} M/l of the butyric (C4:0), caprylic (C8:0), lauric (C12:0), palmitic (C16:0) or stearic acid (C18:0), or with GnRH and 10^{-9} – 10^{-3} M/l of the aforementioned SFA with L-arginine or L-NAME. After 2 or 6 hours of exposure to SFA followed by 2, 6, 12, 18, 24 or 30 h incubation, the media for LH and FSH analysis were collected. Concurrently, NO release and the proliferation index of control and treated cells were determined. There was found that all used SFA reduce GnRH-induced LH and FSH secretion from pituitary cells *in vitro*. The most significant ($P \leq 0.05$) suppressive effect was observed after 6 h exposure of cells to 10^{-3} M/l of caprylic acid, 10^{-4} M/l of palmitic acid and 10^{-4} M/l of stearic acid compared to positive control. SFA did not change significantly NO release. There was no correlation between gonadotropin secretion and NO release under the influence of SFA.

Author for correspondence: Urszula Kosior-Korzecka (urszula.korzecka@up.lublin.pl)

DIETARY SUPPLEMENTATION WITH NETTLE INDUCES APOPTOSIS AND AFFECTED FOLLICULOGENESIS IN THE RABBIT OVARY

K. KAMINSKA¹, K. KAPUSTA¹, S. PALKA², M. KMIECIK², J. ZUBEL-LOJEK³, M. GRZESIAK¹

¹Department of Endocrinology, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland, ²Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Krakow, Krakow, Poland, ³Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Krakow, Poland

There is a growing interest in the use of herbs as a part of complementary medicine in the treatment of reproductive disorders in women. Among a plethora of medical plants, special attention has been paid to nettle (*Urtica dioica* L.), which was found to regulate menstrual cycles in women with polycystic ovary syndrome. However, the cellular and molecular mechanism of its action in the ovary is still unclear. To gain insight into this, we examined the effect of nettle on follicle formation, ovarian cell proliferation and apoptosis, and steroid concentrations in the plasma of juvenile rabbits. Animals were divided into two groups (n=10 per each group) and fed with control or 1% nettle-supplemented pellets from 5 to 12 weeks of age. Just after slaughter, one ovary of each rabbit was fixed in 10% buffered formalin for histology, immunohistochemical localization of proliferating cell nuclear antigen (PCNA) and TUNEL assay, while contralateral ovaries were snap frozen for Western blot analysis of PCNA and caspases-9, -8 and -3 protein abundances. Blood samples were collected for the assessment of progesterone, testosterone and estradiol concentrations. The addition of nettle decreased the numbers of primordial ($P=0.015$) and early antral ($P=0.02$) follicles and increased the number of primary ($P=0.04$) ones when compared with the control group. Furthermore, dietary supplementation with nettle resulted in an increased ($P=0.026$) number of atretic follicles among the secondary follicles class that was also confirmed by TUNEL assay. Results from Western blot analysis showed the induction of apoptosis by nettle through activation of caspase-9 ($P=0.047$), caspase-8 ($P=0.022$) and caspase-3 ($P=0.004$), and no effect on proliferation marked by unchanged PCNA protein abundance. The addition of nettle to the diet did not affect plasma steroid concentrations. In conclusion, nettle affected follicle development in the juvenile rabbit ovary in a stage specific manner; it seems to accelerate the initial recruitment of primordial follicles to growth and increase the atresia of secondary follicles. These effects are probably related to changes at the cellular level due to induction of apoptosis through caspase-mediated routes.

Author for correspondence: Kinga Kaminska (kinga.kaminska@doctoral.uj.edu.pl)

CHEMERIN IMPACT ON DIFFERENTIALLY EXPRESSED GENES IN THE ENDOMETRIAL TRANSCRIPTOME OF PIGS DURING PERIIMPLANTATION PERIOD

K. BORS, G. KOPIJ, L. PAUKSZTO, M. KIEZUN, E. RYTELEWSKA, K. KISIELEWSKA, M. GUDELSKA,
K. SZYMANSKA, K. DOBRZYN, E. ZAOBIDNA, J. JASTRZEBSKI, T. KAMINSKI, N. SMOLINSKA

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Chemerin (CHEM) is one of many biologically active proteins secreted by the adipose tissue, involved in the regulation of the energy homeostasis of the organism. In the present study, RNA-Seq was performed to investigate the expression of protein-coding (mRNAs) transcripts in the *in vitro* cultured porcine endometrial slices collected during implantation (days 15 to 16 of gestation) exposed to CHEM (400 ng/ml). To identify the expression profiles of the treated tissues, the cDNA libraries were performed by TruSeq Stranded Total RNA with Ribo-Zero H/M/R Kit (Illumina, San Diego, USA) and the transcriptomes high-throughput sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, San Diego, USA). All reads were trimmed to equal length (120 bp) and mapped to the pig reference genome with ENSEMBL annotation (Sscrofa11.1.99) using the STAR mapper v. 2.7.1a. To identify the differentially expressed genes (DEGs), we applied the DESeq2 method with correction of batch effect using Surrogate Variable Analysis library. In the current study, among all 130 DEGs, 58 were up-regulated and 72 were down-regulated in the CHEM-treated group. DEGs were assigned to 73 functional annotations. The products of above genes take part in many processes, important for the implantation, such as intense tissue remodeling, cell adhesion, angiogenesis, immune response and steroidogenesis. CHEM affects the transcriptomic profile of the porcine endometrium, and in consequence, may influence a proper course of gestation and embryo development.

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Author for correspondence: Kinga Bors (kinga.bors@uwm.edu.pl)

THE EFFECT OF TYPE 1 DIABETES AND HIGH FAT DIET ON THE EXPRESSION OF RECEPTOR FOR ADVANCED GLYCATION END-PRODUCTS (RAGE) IN UTERUS

K. ZGLEJC-WASZAK¹, A. KORYTKO¹, J. WOJTKIEWICZ¹, K. WASOWICZ², J.K. JURANEK¹

¹University of Warmia and Mazury in Olsztyn, School of Medicine, Collegium Medicum,
Department of Human Physiology and Pathophysiology, Olsztyn, Poland, ²University of Warmia and Mazury in Olsztyn,
Faculty of Veterinary Medicine, Department of Pathophysiology, Olsztyn, Poland

The uterus is an essential organ for reproduction in mammals. A growing body of literature has shown that diabetes mellitus (DM) and high-fat diet (HFD) affect female reproductive function. Moreover, DM and HFD is associated with a chronic low-grade inflammatory state that is involved in the development of associated metabolic complications. We hypothesize that hyperglycemic and high fat milieu may activate Advanced Glycation End-Products (AGEs) molecular pathway in mouse uterus. Receptor for Advanced Glycation End-products (RAGE) is involved in multiple processes related to host immunity, vascular regulation, and inflammatory damage in various disease states. Moreover, RAGE is expressed in nearly all tissues in mammals, with large quantities in lungs. The aim of the study was to determine the expression of RAGE protein in uterine tissues harvested from female mice with DM 1 (intraperitoneal injection of 50 mg/kg streptozotocin diluted in PBS for 5 days; n=5) and female mice fed HFD (ssniff EF R/M D12331 mod. - Surwit, ssniff Spezialdiaeten GmbH, Soest, Germany; n=5) or normal diet *ad libitum* for 32 weeks (Labofeed B, Morawski, Poland; n=5). The expression of RAGE was estimated using immunohistochemical (IHC) staining. The presence and intracellular localization of RAGE protein in luminal epithelial, stromal, glandular epithelium and myometrium cells was confirmed. The highest expression of RAGE was observed in uterine tissues of DM 1 mice ($P \leq 0.05$). Moreover, myometrial expression of RAGE was increased in HFD in comparison to control group ($P \leq 0.05$). These data suggest the presence of an altered uterine environment in females with DM 1 and HFD and indicate that elevated uterine level of RAGE may detrimentally impact endometrial and myometrial function during estrous cycle. We might speculate that DM 1 and HFD could modulate local immune system and activate the AGE-RAGE signaling pathway in uterine tissues during estrous cycle.

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Author for correspondence: Kamila Zglejc-Waszak (kamila.zglejc@uwm.edu.pl; kamilazglejc@gmail.com)

AQUAPORINS EXPRESSION IN REPRODUCTIVE TRACT OF THE BULL (BOS TAURUS) CHANGES WITH SEXUAL MATURITY. PRELIMINARY STUDY

P. OBERSKA¹, P. MALKOWSKA¹, M. GRABOWSKA², M. MURAWSKI³,
D. GACZARZEWICZ⁴, A. SYCZEWSKI⁵, K. MICHALEK¹

¹Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology in Szczecin, Szczecin, Poland, ²Department of Histology and Developmental Biology, Pomeranian Medical University, Szczecin, Poland, ³Department of Animal Nutrition, Biotechnology and Fisheries, University of Agriculture in Krakow, Krakow, Poland, ⁴Department of Animal Reproduction, Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Szczecin, Poland, ⁵Genetic and Animal Husbandry, Szczecin, Poland

Aquaporins (AQPs) also known as water channels (WCPs) are small (28-37 kDa), transmembrane, hydrophobic proteins, that facilitate the transport of water and other small molecules. To date, 13 aquaporins (AQP0-AQP12) have been discovered in mammals, and their presence has been found in a wide range of cell types that build the whole body. Almost all AQPs, except AQP6 and AQP12, are expressed in the male reproductive organs (testis, epididymis, vas deferens) and sperm. Numerous studies have suggested that these proteins are involved in a number of processes responsible for the proper functioning of the male reproductive system and the production of reproductive cells. Reproduction methods are associated with a constant need to search for new factors that not only significantly affect reproductive processes, but also create new possibilities in the assessment of male reproductive potential. Owing to the potential importance of AQPs in the development, maturation and function of male germ cells and in the production of high-quality semen, we have attempted to identify and analyze the expression of AQPs in the male reproductive system of cattle. The experiment is conducted on the Polish male Holstein-Friesian, black and white animals. The study is carried out on the bovine tissues of the male reproductive tract. The tissue samples are collected from three age groups of animals: (i) calves aged 5 to 7 weeks; (ii) young cattle aged 5 to 6 months and (iii) adult bulls aged 1–3 years. To determine the immunolocalization and immunoexpression of AQPs immunohistochemistry and Western blotting are used. So far, it has been revealed that in AQP1 is found in endothelial cells of blood vessels in the bovine testis. On the basis of our preliminary studies, it has been shown that AQP3, AQP7, AQP8 and AQP9 are located within the seminiferous tubules epithelium in cattle. Moreover, expression of AQP3 and AQP7 increase with the age in the bovine testis. The obtained preliminary results seem to be very promising and suggest that AQPs are involved in the proper development of organs and the course of reproductive processes in male cattle.

Author for correspondence: K. Michalek (kmichalek@zut.edu.pl)

IMPACT OF FETAL NUMBER ON ACUTE PHASE PROTEINS, CORTISOL AND HEMATOLOGICAL PARAMETERS IN EWES DURING THE PERIPARTURIENT PERIOD

MONIKA GREGULA-KANIA¹, U. KOSIOR-KORZECKA², K. KANIA³, A. HAHAJ-SIEMBIDA¹

¹Institute of Animal Breeding and Biodiversity Conservation, Faculty of Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Lublin, Poland, ²Sub-Department of Pathophysiology, Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Lublin, Poland, ³Department of BioPhysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Lublin, Poland

An optimal transition requires a comprehensive understanding of the physiological events that occur during the periparturient period. Thus, the appropriate range of maternal APP concentrations should be determined to reflect the response to changes in homeostasis or disease as well as to reduce perinatal mortality. The objective of the study was to compare the plasma concentrations of SAA, Hp, Fb, and cortisol in healthy single- and twin-bearing ewes from 2 weeks before to 2 weeks after parturition. Moreover, pregnancies with more than one fetus are often accompanied by hematological disorders compared to single pregnancies. Thus, we determined hematological parameters in peripheral blood during the periparturient period to assess whether anemia develops and whether twin-lambing sheep are more sensitive to anemia during late pregnancy. We selected only healthy sheep and enrolled a total of 40 ewes of the prolific meat line BCP. The blood samples were obtained by jugular venipuncture and collected in sterile vacuum tubes, with EDTA as an anticoagulant, 14 and 7 days before parturition, a few hours after parturition, and finally 7 and 14 days after parturition. The ewes were classified into two research groups: females with single and twin pregnancy. We measured SAA, fibrinogen, haptoglobin and cortisol concentrations as well as hematology was performed. We found a greater concentration of SAA, Hp, Fb, and cortisol in the periparturient period in twin- compared to single-bearing ewes. There were no differences between single- and twin-bearing ewes for any hematological parameters. The profile of APP changes was similar both in single- and twin-bearing females: an increase in SAA and Fb and a decrease in Hp concentrations. The cortisol concentration did not change significantly. With regard to hematological parameters, both single- and twin-bearing ewes exhibited trends typical for the periparturient period. The values of all parameters were within the physiological range.

Author for correspondence: Monika Gregula-Kania (gregulakania@gmail.com)

CHEMERIN EFFECT ON STEROIDOGENESIS IN THE PORCINE UTERUS: AN *IN VITRO* STUDY

M. GUDELSKA, K. DOBRZYN, E. RYTELEWSKA, K. KISIELEWSKA, M. KIEZUN, E. ZAOBIDNA,
K. BORS, G. KOPIJ, K. SZYMANSKA, B. KAMINSKA, T. KAMINSKI, N. SMOLINSKA

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Steroidogenesis is one of the most important processes which occur in the female reproductive system during both the oestrous cycle and early gestation. In pigs, besides the ovaries and placenta, steroidogenesis process takes also place in the endometrial and myometrial tissues. Chemerin belongs to the family of adipokines - hormones secreted by the adipose tissue. Chemerin exerts pleiotropic effects. Its receptors have been found to be present in the porcine uterus, which suggests that this organ may be sensitive to the adipokine. Additionally, a growing body of evidence indicates the participation of chemerin in the regulation of reproductive system functions. The aim of this study was to determine the influence of chemerin (100 and 200 ng/mL) on steroidogenesis in the porcine endometrium, especially on the secretion of oestradiol (E_2), as well as on the expression of aromatase ($P450_{arom}$) and 3 beta-hydroxysteroid dehydrogenase ($3\beta HSD$) proteins during early gestation: on days 10 to 11 (transuterine migration of embryos) and 12 to 13 (maternal recognition of pregnancy). Concentrations of E_2 in media were defined by radioimmunoassay, whereas the expression of proteins was determined by Western blot method. To analyse statistical differences, one-way ANOVA, followed by Duncan's *post hoc* test, was used ($n=5$). Obtained results showed that chemerin affects production of E_2 in the porcine uterus. On days 10 to 11 of pregnancy, chemerin enhanced the expression of both steroidogenic enzymes and E_2 secretion. However, on days 12 to 13 of gestation, chemerin reduced $P450_{arom}$ and $3\beta HSD$ proteins concentrations in the *in vitro* incubated endometrial slices and E_2 levels in media. The presented data indicate that uterine steroidogenesis in pigs may be dependent on chemerin presence. Moreover, the adipokine influence on E_2 production during crucial stages of pregnancy indicates that chemerin may be an important link between energy homeostasis, immunological processes and reproductive functions in pigs.

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Author for correspondence: Marlena Gudelska (marlena.gudelska@uwm.edu.pl)

THE IMPACT OF CHEMERIN ON THE SECRETION OF CYTOKINES (INTERLEUKIN-1 β , -6) AND THE EXPRESSION OF CYTOKINE RECEPTORS (IL1R1, IL6R) BY THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND EARLY PREGNANCY

G. KOPIJ, M. KIEZUN, E. ZAOBIDNA, K. DOBRZYN, K. KISIELEWSKA, E. RYTELEWSKA,
M. GUDELSKA, K. BORS, K. SZYMANSKA, B. KAMINSKA, T. KAMINSKI, N. SMOLINSKA

University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Chemerin belongs to the group of adipokines involved in the regulation of energy homeostasis. Recent studies have demonstrated that chemerin participates in the regulation of the uterus. In the period of uterine receptivity, the endometrium produces many factors, i.a. cytokines, which are crucial for the proper course of the implantation process. The aim of this study was to investigate the impact of chemerin on the protein expression patterns of cytokine receptors (IL1R1, IL6R). We also aimed to examine the secretion of cytokines (IL-1 β , IL-6) by the porcine endometrium under the influence of chemerin (100, 200 ng/mL). On days 10 to 11 of the oestrous cycle, chemerin decreased the expression of IL1R1. On days 12 to 13 of pregnancy, chemerin at the dose of 200 ng/mL decreased the expression of IL1R1 protein. Moreover, chemerin at the dose of 100 ng/mL caused an increase in IL6R protein expression on days 10 to 11 of the oestrous cycle. The stimulatory influence on IL6R protein expression was observed on days 12 to 13 of pregnancy after treatment with the adipokine at the dose of 200 ng/mL. On days 10 to 11 of the oestrous cycle the adipokine (200 ng/mL) stimulated the secretion of IL-1 β and IL-6. On days 12 to 13 of pregnancy, the adipokine of the dose of 100 ng/mL enhanced, while at the higher dose depressed IL-1 β release. Secretion of IL-6 by the endometrial tissues explant was decreased by chemerin on days 12 to 13 of pregnancy. In conclusion, the influence of chemerin on IL1R1, IL6R protein expression as well as IL-1 β , IL-6 secretion by endometrial explants was dependent on the dose of the adipokine and stage of the oestrous cycle or early pregnancy. Our results imply that chemerin may play an important role in the process of implantation in the pig.

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Author for correspondence: Grzegorz Kopij (grzegorz.kopij@uwm.edu.pl)

ADIPONECTIN AS A PROINFLAMMATORY FACTOR IN THE PORCINE ENDOMETRIUM DURING THE OESTROUS CYCLE AND IMPLANTATION: AN *IN VITRO* STUDY

M. KIEZUN, K. DOBRZYN, E. RYTELEWSKA, K. KISIELEWSKA, M. GUDELSKA, K. BORS, G. KOPIJ,
K. SZYMANSKA, E. ZAOBIDNA, B. KAMINSKA, T. KAMINSKI, N. SMOLINSKA

Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology,
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Adiponectin (ADPN) and its receptors are expressed in the human and porcine uterus. They play an important role in the regulation of reproductive processes. We have previously reported that the adipokine affects uterine steroidogenesis and prostaglandin secretion. These, with addition to the results of our preliminary studies of *in vitro* adiponectin effects on the porcine endometrial proteome with the use of mass spectrometry, prompt us to examine ADPN role in the regulation of immunological processes occurring in this tissue. We hypothesized that ADPN influences endometrial secretion of interleukins 1 β (IL-1 β) and 6 (IL-6), as well as their receptors, IL1R1 and IL-6R, protein expression. Porcine endometrial tissues were obtained from sows on days 10 to 12 of the oestrous cycle (mid-luteal phase, characterized by the highest secretion of progesterone by corpus luteum) and on days 15 to 16 of pregnancy (beginning of implantation). Endometrial explants were preincubated for 2 hours, and subsequently, incubated for another 24 h with ADPN (10 μ g/mL) or without any treatment (control). Enzyme-linked immunosorbent assay (ELISA) method was used to evaluate the concentration of IL-1 β and IL-6 in the culture media, whereas Western blot method was employed for examination of IL-1R1 and IL-6R protein content in the endometrial tissue. We have observed a stimulatory effect of ADPN on IL-1 β and IL-6 secretion, as well as on IL1R1 and IL-6R protein content in all the examined tissues, except for endometrial explants from days 10 to 12 of the oestrous cycle in which ADPN caused a decrease in IL-1 β release. The obtained results suggest that ADPN may be an important factor regulating immunological processes in the endometrium both during the oestrous cycle and at the stage of implantation. Proinflammatory action of ADPN in this tissue, especially at the window of implantation, may affect the embryo-maternal communication and, as a result, embryo adhesion and implantation.

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Author for correspondence: Marta Kiezun (marta.kiezun@uwm.edu.pl)

THE INFLUENCE OF KETOGENIC DIET ON THE COURSE OF GESTATION AND BIOCHEMICAL COMPOSITION OF HIPPOCAMPAL FORMATION OF PREGNANT RATS

Z. RAUK¹, P. SZULC², W. KOSIEK¹, Z. SETKOWICZ-JANECZKO¹

¹Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Faculty of Biology,
Jagiellonian University, Krakow, Poland, ²Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University,
Krakow, Poland

Ketogenic diet (KD) is a high-fat, low-carbohydrate diet, used in the treatment of drug-resistant epilepsy, both in children and in adults, including pregnant women. The main goal of this research was to determine the influence of KD application on the course of gestation and the biochemical composition of the brain of pregnant female rats. The animals were divided into 2 groups, obtaining normal or ketogenic diet. The body weight, blood level of glucose and ketone bodies and food consumption were monitored during the gestation period. After the delivery the number, sex and body weight of pups was assessed. The brain tissue of females was analyzed using FTIR method, in order to assess the biochemical composition of the hippocampal formation. The statistical analysis revealed a significantly higher ketone bodies blood level since the 4th day of gestation and a lower glucose level in 20th day of gestation in KD fed rats than in control group. Both groups presented a similar pattern of weight gaining during pregnancy, brain weight to body weight ratio in 2nd day postpartum, number of pups and the sex ratio of the brood. However, the pups' weight was significantly lower in KD fed group, despite the fact that KD fed rats appeared to consume significantly more calories per day. The pups' weight correlated negatively with mother's ketone bodies blood level and positively with glucose blood level in 20th day of gestation. The obtained results indicate a negative influence of ketosis on the birth weight and a greater importance of diet composition than calorificity in fetus development. The preliminary analysis of FTIR spectra for bands 1740cm⁻¹, 1360–1480 cm⁻¹ and 2924 cm⁻¹ (ketone bodies, unsaturated fatty acids, saturated fatty acids) revealed no differences between ketogenic and normal diet fed rats.

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Author for correspondence: Zuzanna Rauk (zuzanna.rauk@student.uj.edu.pl)

VISFATIN GENE EXPRESSION IN THE PORCINE PITUITARY GLAND DURING THE OESTROUS CYCLE AND EARLY PREGNANCY

K. SZYMANSKA¹, M. KIEZUN¹, E. ZAOBIDNA¹, K. DOBRZYN¹, E. MLYCZYNSKA², E. RYTELEWSKA¹, K. KISIELEWSKA¹, M. GUDELSKA¹, K. BORS¹, G. KOPIJ¹, B. KAMINSKA¹, A. RAK², N. SMOLINSKA¹, T. KAMINSKI¹

¹Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,

²Department of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland

Visfatin appears to be an energy sensor involved in the regulation of female fertility, which creates a hormonal link integrating the control of energy homeostasis and reproduction. Visfatin expression in adipocytes can be affected by hormonal factors such as steroid hormones, tumor necrosis factor- α , growth hormone and dexamethasone, while in the human granulosa cells by human chorionic gonadotropin and prostaglandin E₂. It is suggested that visfatin gene expression can be controlled by species-specific regulatory mechanism and the adipokine concentrations in human adipose tissue are affected by hormonal status related to pregnancy. We hypothesized that hormonal milieu connected with the specific phase of the oestrous cycle affects visfatin gene expression in the porcine pituitary. The gene expression was evaluated by RT-PCR method. Data were analysed based on one-way ANOVA and LSD *post hoc* test. During the oestrous cycle, the highest expression of visfatin gene was observed on days 10–12 and the lowest on days 17–19. During pregnancy, visfatin gene expression was the highest on days 12–13 and 27–28 in comparison to days 15–16. Comparing visfatin gene expression throughout the early pregnancy with days 10–12 of the oestrous cycle, visfatin mRNA content during all periods of pregnancy was significantly lower. The fluctuations in the expression levels of visfatin noticed during the oestrous cycle and early pregnancy may suggest its dependence on the hormonal milieu specific for the oestrous cycle and early pregnancy.

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