

# Polish-French symposium on new regulations of reproduction: the role of adipokines

20/10/2022

*Académie Polonaise des Sciences; Centre Scientifique à Paris; 74, rue Lauriston; 75116 Paris*

## SYMPOSIUM BOOK



Organizers



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JAGIELLONIAN UNIVERSITY  
IN KRAKÓW

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JAGIELLONIAN UNIVERSITY  
IN KRAKÓW

Polish Academy of Sciences, Scientific Center in Paris



Polish Society for the Study of Reproduction



## Organising Committee:

dr hab. Agnieszka Rak (Jagiellonian University in Kraków, Poland)

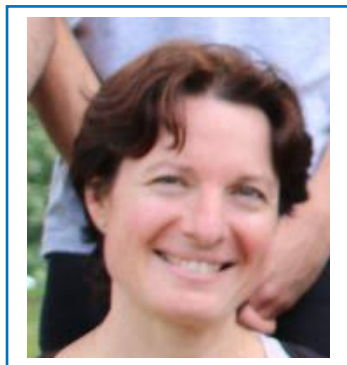
dr Joëlle Dupont (INRAE, France)

Diana Kogaczewska (Polish Academy of Sciences Scientific Center in Paris, France)

## INTRODUCTION

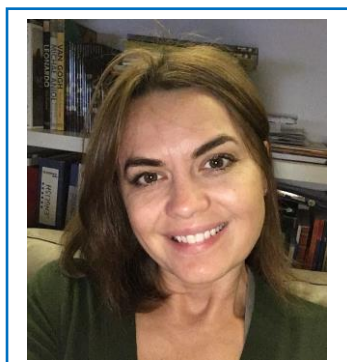
The increase in infertility and its social costs are the starting point for a global debate on the search for optimal systemic solutions that increase the chances of improving the effectiveness of infertility prevention and treatment. Current research focuses on the discovery and description of new fertility biomarkers and a better regulation of reproductive functions to increase fertility in animal and human species. It is well known that adipose tissue can influence puberty, sexual maturation, and fertility in different species. Adipose tissue secretes molecules called adipokines which most likely have an endocrine effect on reproductive function. It has been shown over the last few years that adipokines are functionally implicated at all levels of the reproductive axis including the gonad and hypothalamic-pituitary axis. Many studies have demonstrated the presence and the role of the adipokines and their receptors in the female reproductive tract of different species. These adipokines regulate ovarian steroidogenesis, oocyte maturation, and embryo development. They are also present in the uterus and placenta where they could create a favorable environment for embryonic implantation and play a key role in maternal-fetal metabolism communication and gestation. Reproductive functions are strongly dependent on energy balance, and thereby metabolic abnormalities can lead to the development of some pathophysiology such as polycystic ovary syndrome (PCOS) in women. Adipokines could be a link between reproduction and energy metabolism and could partly explain some infertility related to obesity or PCOS. The topics of this meeting will be focused on the role of adipokines in central (hypothalamus – pituitary) and peripheral (gonads, uterus, placenta) regulation of fertility under normal and pathological conditions. The symposium will also provide an opportunity to discuss the practical application of current knowledge about adipokines in the reproductive system. A particular focus will be on cooperation between bio and physiologists, veterinarians and gynaecologists from Poland and France in a broader international context.

Joelle Dupont & Agnieszka Rak



**JOËLLE DUPONT, PhD**  
**NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE, FOOD AND THE ENVIRONMENT, FRANCE**

Joëlle, Director of Research at UMR PRC (Joint research unit Physiology of Reproduction and Behavior) INRAE (National Research Institute for Agriculture, Food and the Environment) Centre Val Loire, Nouzilly, is an expert on the molecular mechanisms of adipokines in reproductive functions. She received her M.S., Ph.D., and HDR in France in 1999 and she spent two years at NIDDK, NIH, Bethesda, in USA for her Post-doc. She is internationally recognized in the links between metabolism and reproductive functions in human and domestic animal models. Her lab has reported main advances in major recognized reproductive journals such as Human Reproduction, Reproduction and Biology of Reproduction. Joëlle authored more than 200 papers in international journals, 250 abstracts and 5 book chapters and was PI of several National and European projects. Her work has been cited 11,667 times (h index 58 in ResearchGate) and she trained more than 30 young scientists (M.S and Ph.D students). She served on panels for National Science Center Poland (NCN), Science Foundation of Ireland, National Research Foundation of Korea and reviewer for various journals, 3 Editorial boards (Reproduction, Frontiers in Endocrinology, and Livestock Science). Since 2021, she is deputy director of the UMR PRC and member of the board of the doctoral school SSBCV (Health, Biological Sciences and Chemistry of Life) of the University of Tours.



**PROFESSOR AGNIESZKA RAK,**  
**JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

Agnieszka works as an associate professor at the Jagiellonian University in Kraków. She received her PhD in 2008 and HDR in 2016 in Poland. She joined international labs in Japan, Portugal, Germany and France. She is a leader of the research team at Jagiellonian University investigating the role of metabolic hormones and adipokines in the regulation of the female reproduction (hypothalamus, pituitary, ovary and placenta cells) in human, rodents and domestic pig. She is an active member in international and national organisations, e.g. of European Society for Domestic Animals (ESDAR) and Polish Society for Biology of Reproduction, Polish Society of Physiology and Polish Academy of Sciences. Agnieszka authored more than 92 papers in international journal, 140 abstracts, and 3 book chapters. Her work has been cited 1,328 time with an h-index of 22. Agnieszka was PI/research supervisor of 10 projects (e.g. Iuventus Plus, SONATA, HARMONIA, OPUSes, SONATA BIS) and a main investigator in 6 other projects. She supervised 2 PhD theses (5 in progress) and about 45 master/bachelor's theses. She served on panels NCN, Austrian Science Fund, the USA-Israel Agricultural Research & Development Fund, Slovak Academy of Sciences and French Le Studium Loire Valley Institute for Advanced Studies and is an internationally recognized reviewer for various journals. Agnieszka was awarded the Foundation for Polish Science "START", Scholarship of the Minister of Science and Higher Education for Young Prominent Scientist and she received Brown Cross of Merit.

## INVITED SPEAKER

We are extremely honored and happy to introduce our superb Invited Speaker:



**PROFESSOR SAMI DRIDI**  
**UNIVERSITY OF ARKANSAS, USA**

Sami, Professor of Avian Endocrinology and Molecular Genetics in the Department of Poultry Science at the University of Arkansas, is one of the pioneer researchers who investigates the mode of action of feed additives on growth efficiency of poultry under both standard and heat stress conditions. He received his M.S., Ph.D., and HDR in France. He served as a quality inspector in poultry industry, and he joined several international labs as postdoc/PI such as UNC Chapel Hill, UK, WVU, KUL Belgium, ENITAB and ENVN France. Sami is an internationally recognized scientist who has pioneered innovative concepts in poultry research and molecular nutrition. His lab has reported seminal advances in high ranked journals such as J Nutr, J Anim Sci, Am J Pathol and Am J Physiol. He developed a strategy to isolate dsRNA from animal tissues, and identified a key role of Dicer1 in lameness in broilers. He also defined the mechanisms by which HyD enhances breast muscle yield in broiler. He also defined the peripheral and central molecular mechanisms involved in the beneficial effects of phytogenics and quantum blue supplementation under heat stress conditions and in woody breast myopathy, respectively. He identified GRP75 as a new molecular signature for heat stress and developed feather-HSP70 as a non-invasive method to monitor stress in poultry. In collaboration with more than 500 authors, he published the 3<sup>rd</sup> and the 4<sup>th</sup> set of guidelines for standardizing research in autophagy. Sami authored more than 121 papers, 122 abstracts, 10 book chapters, one book, and co-edited the avian physiology book edition 7. His work has been cited 11,020 time with an h-index of 29. He advised more than 26 scientists, and served in 50 advisory committees. He served on panels for NSF and DFG, reviewer for 74 journals, 3 Editorial boards, and he is a member of PSA and the President of WPSA-USA branch.



## PROGRAMME

08:45-09:00 REGISTRATION

09:00-09:10 WELCOME SPEECH

Magdalena Sajdak (Polish Academy of Sciences Scientific Center in Paris)  
Joelle Dupont (INRAE) & Agnieszka Rak (Jagiellonian University in Kraków)

**FIRST PANEL:** *The role of adipokines on fertility regulation at central level:  
hypothalamus – pituitary*

**Moderators:** Tadeusz Kamiński (University of Warmia and Mazury in Olsztyn)  
Anthony Estienne (INRAE)

09:10-09:25 Małgorzata Szczęśna (University of Agriculture in Kraków)  
*Pregnancy – induced adaptation of central sensitivity to leptin – lesson from large animal model.*

09:25-09:40 Laurence Dufourny (INRAE) **ONLINE**  
*Does apelin interact with GnRH and kisspeptin neuronal populations to modulate the central control of reproduction in male rodents? A neuroanatomical, molecular and functional investigation.*

09:40-09:55 Kamil Dobrzyń (University of Warmia and Mazury in Olsztyn)  
*Adipokines, important agents in the regulation of the hypothalamic-pituitary-ovarian axis.*

09:55-10:10 DISCUSSION

10:10-10:30 COFFEE BREAK & POSTER SESSION I

Małgorzata Kotula-Balak (University of Agriculture in Kraków)  
*Adipokine signaling in health and disease – studies in human, dog, boar and rodent testes.*

Agnieszka Partyka (Wrocław University of Environmental and Life Sciences)  
*Spermatozoal functions and characteristics – assessment tools in obesity.*

Weronika Biernat (University of Agriculture in Kraków)  
*The roles of leptin and resistin in reproduction and leptin resistance in sheep.*

Karolina Pich (Jagiellonian University in Kraków)  
*Effect of omentin on steroid synthesis in porcine ovarian follicles. In vitro study.*

Natalia Respekta (Jagiellonian University in Kraków)  
*Expression and action of omentin on porcine pituitary cell function.*

Ewa Mlyczyńska (Jagiellonian University in Kraków)  
*Visfatin in the porcine corpus luteum: expression and action on steroid synthesis and angiogenesis.*

Dominika Wachowska (Jagiellonian University in Kraków)  
*Asprosin – new adipokine in female reproduction.*

**SECOND PANEL: The role of adipokines on fertility regulation at peripheral: ovary & testis**

**Moderators:** Wojciech Nizański (Wrocław University of Environmental and Life Sciences)  
Pascal Froment (INRAE)

10:30-10:45 Cecilia Dall'Aglio (University of Perugia)  
*Adipokines expression in ewes subjected to the influence of different nutritional level.*

10:45-11:00 Patrycja Kurowska (Jagiellonian University in Kraków)  
*Expression and role of spexin in human granulosa cells.*

11:00-11:15 Anthony Estienne (INRAE)  
*Chemerin produced locally within the reproductive tract impairs testicular function in roosters.*

11:15-11:30 Guillaume Bourbon (INRAE) **ONLINE**  
*The hepatokine FGF21 increases the human spermatozoa motility.*

11:30-11:45 Mathilde Daudon (INRAE) **ONLINE**  
*Fibronectin Type III domain containing 5 (FNDC5) expression in bovine ovary and in vitro effects on bovine granulosa cells proliferation and steroidogenesis.*



11:45-12:00 DISCUSSION

12:00-13:30 LUNCH BREAK

13:30-14:00 Invited speaker Sami Dridi (University of Arkansas) **ONLINE**  
*Role of avian orexin in muscle energy metabolism and hepatic lipogenesis.*

14:00-14:10 DISCUSSION

**THIRD PANEL:** *The role of adipokines on fertility regulation at peripheral: uterus, placenta, embryo development & pregnancy*

**Moderators:** Nina Smolińska (University of Warmia and Mazury in Olsztyn)  
Joelle Dupont (INRAE)

14:10-14:25 Marta Kieżun (University of Warmia and Mazury in Olsztyn)  
*Adipokines, rising stars in early pregnancy theater.*

14:25-14:40 Monika Dawid (Jagiellonian University in Kraków)  
*Visfatin in human placenta cells.*

14:40-14:55 Marta Hita Hernandez (INRAE-BREED)  
*Role of adiponectin on placental sphingolipid metabolism in maternal obesity.*

14:55-15:10 Ophélie Bernardi (INRAE) **ONLINE**  
*Chemerin in egg albumen and cell number of germinal disc: potential biomarkers of the embryo development for genetic selection in birds.*

15:10-15:25 DISCUSSION

15:25-15:45 COFFEE BREAK & POSTER SESSION II (ONLINE)

Marlena Gudelska (University of Warmia and Mazury in Olsztyn)  
*The role of chemerin in the uterus of domestic pig (*Sus scrofa domestica* L.) during the oestrus cycle and early pregnancy.*

Grzegorz Kopij (University of Warmia and Mazury in Olsztyn)  
*Visfatin impact on the transcriptome luteal cells of domestic pig (*Sus scrofa**

*domestica L.) during early pregnancy.*

Loise Serra: *Effect of endocrine disruptors on adipokine secretions.*

**FOURTH PANEL:** *Polish-French science possibility and future plan*

15:45-17:00 Agnieszka Rak & Joelle Dupont

## FIRST PANEL

*The role of adipokines on fertility regulation at central level: hypothalamus – pituitary*

**Moderators:** Tadeusz Kamiński & Anthony Estienne



**PROFESSOR TADEUSZ KAMIŃSKI**

**UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND**

Tadeusz is a professor of natural sciences at the Faculty of Biology and Biotechnology of the University of Warmia and Mazury in Olsztyn, Poland. In the years 2011-2019 he was the dean of this faculty, currently he is the member of the University Council. He examined the participation of opioid peptides in the regulation of pig ovarian functions, including local opioid production, their influence on ovarian steroidogenesis and the mechanism of action in ovarian cells. For over ten years, prof. Kamiński is involved in the study of the role of adipokines in the pig reproductive system during the oestrus cycle and early pregnancy (critical period for the survival of embryos). Using, apart from classical research tools for endocrinology, also molecular, transcriptomic and proteomic studies, he found the expression of a number of adipokines and their receptors in the structures of the hypothalamic-pituitary-ovarian axis, responsible for the control of the reproductive system, in the ovaries (luteal cells, granulosa and theca cells), the uterus and trophoblasts. He also detected the influence of adipokines on a number of physiological processes, including production of hypothalamic gonadoliberin, pituitary gonadotropins, steroidogenesis, angiogenesis or apoptosis, essential for the functioning of the reproductive system. Scientific achievements of prof. Kamiński includes, among others over 100 articles, cited over 1,400 times (h-index = 22) and chapters in three books. Professor T. Kamiński was the PI/research supervisor of eight projects (three of them are currently implemented) and a main investigator in eight other projects. For his scientific activity, prof. Kaminski received, among others Individual Award of the Minister of Science and Higher Education and the Award of the President of Olsztyn - the Statuette of St. James, created for the 650<sup>th</sup> anniversary of the city's founding, in the category of science.



**ANTHONY ESTIENNE, PhD**  
**POSTDOCTORAL RESEARCHER**  
**NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE, FOOD AND THE ENVIRONMENT, FRANCE**

Anthony is a postdoctoral researcher in the SENSOR laboratory at INRAE in Tours in France, specialized in the ovarian physiology of several species of agronomic interest. He has also worked on human reproductive physiology projects. He worked during his thesis under the supervision of Dr Danièle Monniaux from INRAE in Tours on ovarian physiology and more specifically the regulation of the Anti-Müllerian Hormone (AMH) system by Bone Morphogenetics Proteins (BMPs) and its consequence on the ovarian phenotype in several study models. Subsequently, he continued his academic training with a post-doctoral internship in Canada in the laboratory of Prof. Christopher A Price of the University of Montreal in Saint-Hyacinthe, which focused on follicular atresia and ovulation. Finally, he is currently pursuing a second post-doctorate in France in the laboratory of Joëlle Dupont at INRAE in Tours with research projects focusing on the relationship between metabolism and reproduction, particularly in chickens and humans. Recently, he became interested in the effects of certain phytosanitary products (herbicides) on reproduction in the species above mentioned. Understanding the reproductive function and its physiology dictated his academic and professional choices during his career.

## ORAL PRESENTATION I



**PROFESSOR MAŁGORZATA SZCZĘSNA**  
**UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND**

Małgorzata works as an associate professor at the University of Agriculture in Krakow at the Department of Animal Nutrition and Biotechnology, and Fisheries (Faculty of Animal Science). She investigates the mechanisms that determine the occurrence of pregnancy-induced leptin resistance and studies the accompanying processes that affect the physiology of pregnant and lactating sheep. Previously she has conducted researches focused on seasonal leptin resistance in sheep. Her main interest is to comprehensively understand the phenomenon of leptin resistance, including the causes of its emergence and its effects on the organism. Apart of that she investigated interactions between other hormones involved in the regulation of metabolism and sheep reproduction (such as ghrelin, orexin, resistin, thyroid hormones, prolactin, growth hormone, melatonin, follicle-stimulating and luteinizing hormones). The main aim of the research that she conducted is to explain the endocrine mechanisms that allow sheep to optimally adapt to changing energy requirements of the organism and the varied possibilities of obtaining energy. She conducted experiments using *in vitro* tissue and cell culture as well as *in vivo* animal studies. She authored more than 31 papers, 90 abstracts, 1 book chapter. Her papers have been cited more than 260 time with an h-index of 9. She co-supervised one doctoral dissertation and supervised 24 other diploma thesis.

## Pregnancy - induced adaptation of central sensitivity to leptin - lesson from large animal model

SZCZESNA M, BIERNAT W, KIRSZ K, ZIEBA DA

*University of Agriculture in Krakow, Krakow*

**BACKGROUND:** Pregnancy regulates leptin physiology by affecting its synthesis, availability and activity. Moreover, gestation is a state of hyperphagia that occurs despite coexisting hyperleptinemia; therefore, it is an excellent model for the investigation of phenomenon of leptin-resistance.

**MATERIALS & METHODS:** Experiments were performed using 27 pregnant Polish Longwool ewes. Twelve of them were assigned randomly to receive either saline or recombinant ovine leptin at 2.5 or 5.0 µg/kg body weight. Blood samples for determination of prolactin, growth hormone and melatonin concentration were collected at 15-min intervals over 4 h. Treatments were repeated at 2-wk intervals, starting before mating and continuing for 30-135 days during gestation. Anterior pituitary, mediobasal hypothalamus, arcuate nucleus, median eminence, choroid plexus, and pineal gland were isolated from other non-pregnant ewes and ewes euthanized at 30, 60, 90, 120 d of pregnancy (3 ewes/group) to measure leptin receptors (LR) and SOCS-3 mRNA abundance.

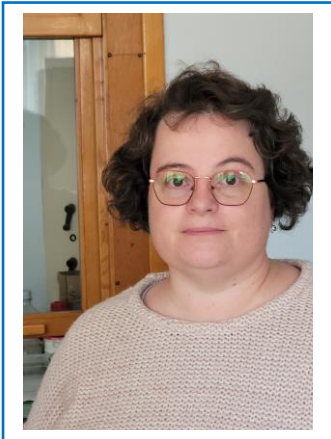
**RESULTS:** It has been shown that the effect of exogenous leptin on the secretion of prolactin, growth hormone and melatonin is dependent on the stage of pregnancy. Also the pattern of expression of LRb and SOCS-3 differs significantly among brain locations and by stage of pregnancy. Results indicate on hypothalamic downregulation of LRb and upregulation of SOCS-3 during ovine pregnancy.

**SUMMARY:** The changes identified during pregnancy in sheep in relation to effects of leptin on hormones secretion and variation in expression of key elements of leptin signaling pathway may be essential for specific pregnancy-induced endocrine adaptations that mediate adjustments in metabolism during gestation.

**Keywords:** leptin, leptin-resistance, pregnancy, sheep

**Funding:** National Science Centre, Poland 2013/09/B/NZ4/01532





**DUFOURNY LAURENCE, PhD**  
**CNRS RESEARCH SCIENTIST**

Laurence received her Master degree, Ph.D in Neurosciences and HDR in France. She studied the central control of ovulation from the start of her career and the neuronal networks driving sexual behavior during her Ph. D and in two postdocs performed at the University of Bristol (UK) and the University of Wyoming (USA). She was hired by CNRS in 2004 and from this time she joined the Department of Physiology of Reproduction and Behavior located in Nouzilly, France. She is a recognized expert in the field of neuroendocrinology and is deeply interested by the influence of endogenous hormones (steroids, melatonin and metabolic hormones) and neuromodulators on neuronal populations driving ovulation and more specifically, kisspeptin and GnRH neurons. Her goal is to unravel the different regulatory pathways and the different components involved in the stimulation/inhibition of kisspeptin release and hence GnRH secretion. She also identified GPR50 as the ortholog of Mel1C receptor a high affinity receptor that lost its ability to bind melatonin through evolution. She is working on the ovine species as it is a well-known model for studying a specific aspect of reproduction, seasonal reproduction, and Laurence Dufourny is also using rodents for studies regarding metabolism. So far Laurence Dufourny published more than forty articles and reviews and gave more than 60 presentations in French and international scientific meetings. Her h-index is 19 and her work has been cited more than 1200 times. She served as General Secretary of the French Neuroendocrine Society (SNE) for 3 years and is still representing the SNE at the French Association of Scientific Societies that gather Societies from all scientific domains.

## Does apelin interact with GnRH and kisspeptin neuronal populations to modulate the central control of reproduction in male rodents? A neuroanatomical, molecular and functional investigation

ABOT A<sup>1</sup>, ROBERT V<sup>2</sup>, FLEUROT R<sup>2</sup>, DARDENTE H<sup>2</sup>, HELLIER V<sup>2</sup>, FROMENT P<sup>2</sup>, DUITTOZ A<sup>2</sup>,  
KNAUF C<sup>1</sup>, DUFOURNY L<sup>2</sup>

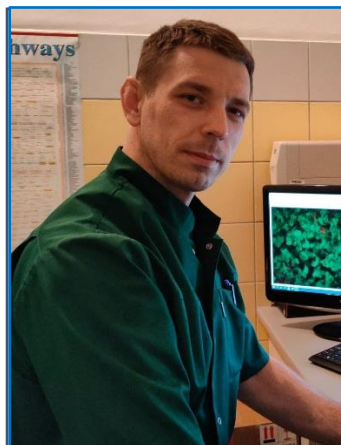
<sup>1</sup> *Institut National de la Santé et de la Recherche Médicale (INSERM), U1220, Université Paul Sabatier, UPS, Institut de Recherche en Santé Digestive et Nutrition (IRSD), CHU Purpan, Place du Docteur Baylac, International Laboratory NeuroMicrobiota, CS 60039, 31024 Toulouse Cedex 3, France* <sup>2</sup> *CNRS, IFCE, INRAE, Université de Tours, PRC, F-37380, Nouzilly, France*

**BACKGROUND:** Hypothalamic control of reproduction relies on GnRH and kisspeptin (KP) secretions. KP neurons are sensitive to sex steroids and metabolic status and their distribution overlaps with neurons producing apelin, a metabolic hormone known to decrease LH secretion in rodents. Previous reports showed an acute effect of apelin on LH secretion in male rats. Our aim here was to unravel the mechanisms induced by apelin occurring at the hypothalamic level and more specifically at the level of KP neurons from the arcuate nucleus and at the level of GnRH neurons distributed more rostrally.

**RESULTS:** Using double immunohistochemical staining and confocal microscopy, we first observed neuroanatomical contacts between apelin fibers and both KP and GnRH neurons in the hypothalamus of male rats and mice suggesting a possible direct action of apelin on the activities of these two populations. We then performed intracerebroventricular apelin infusion for 2 weeks in male mice using Alzet pumps. Chronic apelin treatment did not decrease LH levels nor did it affect gene expression for KP, neurokinin B and dynorphin. Finally, a third experiment was conducted on GnRH neurons cultured from nasal explants. It showed that increasing apelin concentrations did not modulate Ca<sup>2+</sup> levels of cultured GnRH neurons, while 10µM apelin infusion on forskolin pretreated GnRH neurons reevoked a rhythmic activity in 18% of GnRH neurons.

**SUMMARY:** These results suggest that acute apelin effect on LH secretion does not involve modulation of gene expression in KP neurons but may affect the secretory activity of GnRH neurons.

**Keywords:** adipokines, kisspeptin, dynorphin, neurokinin B, hypothalamus, reproduction, rodents.



**KAMIL DOBRZYN, PhD**

**UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND**

Kamil is an Assistant Professor at the Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. He is a member of the Polish Society for Reproductive Biology and the Polish Physiological Society. Kamil completed a 3-month internship at University of Perugia, Italy. He is a member of the research team investigating the role of adipokines and neurohormones in the regulation of the female reproductive system in the domestic pig. During the research for his PhD thesis, defended in 2017, Kamil focused on the role of adiponectin in the regulation of uterine functions during the early gestation period. Kamil participated in 4 completed and 3 ongoing grants supported by NCN of Poland. He received funding and the role of PI for the implementation of a single research task Miniatura-3 supported by NCN. Kamil took an active part in many international conferences, including International Congress on Pig Reproduction or the European Society for Domestic Animal Reproduction Congress. Kamil Dobrzyn is a co-author of 47 scientific papers. His papers have been cited 552 times and his h-index is 14.

## Adipokines, important agents in the regulation of the hypothalamic-pituitary-ovarian axis

DOBRYN K<sup>1,2</sup>, KIEZUN M<sup>2</sup>, KAMINSKA B<sup>2</sup>, ZAObIDNA E<sup>2,3</sup>, RYTELEWSKA E<sup>2</sup>, KISIELEWSKA K<sup>2</sup>,  
GUDELSKA M<sup>2</sup>, ORZECHowSKA K<sup>2</sup>, KOPIJ G<sup>2</sup>, SZYMANSKA K<sup>2</sup>, ZARZECKA B<sup>2</sup>, BIAGINI A<sup>2</sup>,  
SMOLINSKA N<sup>2</sup>, KAMINSKI T<sup>2</sup>

<sup>1</sup>Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland; <sup>2</sup> Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland; <sup>3</sup>Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland

**BACKGROUND:** Reproduction in female mammals belongs to the most energy demanding processes. Due to this fact, the existence of the regulatory link between the reproductive system and energy metabolism is postulated. The adipokines, hormones produced mainly by the white adipose tissue, may be a crucial element of this system. Evidence collected by our research team let us to assume that adipokines may regulate the female reproductive functions through their influence on the hypothalamic-pituitary-ovarian (HPO) axis.

**MATERIALS & METHODS:** Our research focus on the domestic pig as a model for physiological studies on the reproductive system. The analyses are conducted with the use of classic molecular biology methods such as *in vitro* cell cultures, qPCR, Western blotting, RIA and ELISA, as well as using high-throughput methods like Microarrays, NGS and LC-MS.

**RESULTS:** We confirmed the expression of several adipokines and their receptors in all structures of the HPO axis. Their variable expression across the cycle and early gestation indicates adipokines' expression is dependent on the local hormonal milieu. We proved that *in vitro* the adipokines may affect FSH and LH release by the isolated anterior pituitary cells, as well as steroidogenic processes in the ovaries. The adipokines may also affect cell proliferation and apoptosis processes. The high-throughput analyses revealed their influence on the global gene and protein expression in the pituitary and ovarian cells.

**SUMMARY:** The results collected by our team, pertaining to all structures of HPO axis, indicate that adipokines play an important regulatory function in the reproductive system.

**Keywords:** adipokines, oestrous cycle, early gestation, hypothalamus, pituitary, ovary, reproduction, pig

**Funding:** This research was supported by the Polish State Committee for Scientific Research (projects: PBZ KBN-084/P06/2002JP, NN308122134, 0206.0805), Polish National Science Centre (Opus-1 2011/01/B/NZ4/01596, Opus-9 2015/17/B/NZ9/03595, Opus-16 2018/31/B/NZ9/00781, Opus-20 2020/39/B/NZ9/01061, Preludium-14 2017/27/N/NZ9/00638, Preludium-16 2018/31/N/NZ9/00544, Preludium-16 2018/31/N/NZ2/02655).

## POSTER SESSION I



**PROFESSOR MALGORZATA KOTULA-BALAK**  
**UNIVERSITY CENTRE OF VETERINARY MEDICINE AT THE UNIVERSITY OF**  
**AGRICULTURE IN KRAKOW, POLAND**

She works at the University Centre of Veterinary Medicine at the University of Agriculture in Krakow, Poland, and is Head of the Laboratory of Histopathology in the Center for Experimental and Innovative Medicine at the University of Agriculture in Krakow, Poland. Currently, she is charming of Rector Commission for International Cooperation. The main field of her research interest is andrology. She is especially focused on studies on estrogens role in the male reproductive system in various animal species. Her most important scientific achievements are: demonstration for the first time of the presence and role of (i) estrogen synthase (aromatase) in the gonads of seasonal rodents, (ii) non-classical estrogen receptors: estrogen-related receptor (ERR) and membrane estrogen receptor coupled with G protein (GPER) in the gonads of laboratory, farm animals and patients with a Leydig cell tumor and (iii) regulatory cells - telocytes in rodent gonads. She published over 90 original research articles (h-index= 18). She was a post-doc at Adelaide University, Adelaide, Australia, and at the National Institutes of Health, Bethesda, United States of America. She was a PI on NCN projects e.g. SONATA BIS5 and OPUSes. She is a Secretary of the Main Board of the Society for Reproductive Biology, a Member of the Biology Committee of the Polish Academy of Sciences, a Member of editorial boards of international scientific journals, and an Editor of special issues.

## Adipokine signaling in health and disease - studies in human, dog, boar, and rodent testes

KOTULA-BALAK M<sup>1</sup>, PAWLICKI P<sup>2</sup>, RAMISZ G<sup>1</sup>, RAK A<sup>3</sup>, TARASIUK K<sup>1</sup>

<sup>1</sup> University Centre of Veterinary Medicine JU-UA, University of Agriculture in Krakow, Poland Jagiellonian University, Krakow; <sup>2</sup>Center of Experimental and Innovative Medicine, University of Agriculture in Krakow, Poland; <sup>3</sup>Laboratory of Physiology and Toxicology, Jagiellonian University in Krakow, Poland

**BACKGROUND:** Lipid homeostasis is crucial for the function of reproductive organs and organism health. However, not much is known about the distinct role of adipokines in the male both in physiological and pathological conditions.

**MATERIALS & METHODS:** Testes were obtained from (i) mature bank voles subjected to long and short photoperiod conditions and treated with G-protein coupled estrogen receptor (GPER) antagonist, (ii) mature Landrace boars - Improvac immunocastrated [gonadoliberin (GnRH) analog], (iii) mature mixed-breed dogs with cryptorchidism or germ cell tumor. (iv) Biopsies with Leydig cell tumors were obtained from 29-34-year-old patients. The material was analyzed for expression of adiponectin and leptin with qRT-PCR, western blotting, immunohistochemistry, and Elisa immunoassays.

**RESULTS:** We revealed photoperiod- and estrogen-GPER-dependent expressions of adiponectin and leptin in bank vole testis associated with spermatogenesis activity. In the above conditions, expressions of adipokines were decreased. In immunocastrated boar testis, GnRH-regulated expressions of these adipokines were associated with an increased level of cholesterol. In canine, both cryptorchid and tumor testis, signalings of both adipokines were decreased together with mainly decreased expression of estrogen-related receptors. On the other hand, in Leydig cell tumors, signalings of both adipokines were increased and correlated with lipid droplet increase and high estrogen level.

**SUMMARY:** In the testis, adiponectin and leptin are regulated by a physical factor (photoperiod) and hormones (GnRH, estrogen). Their expressions/signalings are associated with spermatogenesis activity (bank vole, boar), its perturbation (boar, dog, and human), and fattening (boar). Further studies on adipokines as potential markers of the above processes are needed.

**Keywords:** Leydig cell, spermatogenesis, adiponectin, leptin, photoperiod, immunocastration, cryptorchidism, tumor

**Funding:** supported by a grant SUB/2020-080100-D016 as part of the statutory activities from the Ministry of Education and Science to the University Centre of Veterinary Medicine, University JU-UA, Agriculture in Krakow.





**PROFESSOR AGNIESZKA PARTYKA**

**WROCLAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCE, POLAND**

Agnieszka was graduated in biology and defended her PhD thesis titled *“The effect of cryopreservation on lipid peroxidation and antioxidant balance in avian semen”* at Wroclaw University in 2009 receiving the title of doctor of biology (specialist in biotechnology). She became a member of the team at Department of Reproduction and Clinic of Farm Animals, in Wroclaw University of Environmental and Life Science in 2010, and obtained habilitation in discipline veterinary in 2018. Since 2020 she is associate professor at the Department of Reproduction and Clinic of Farm Animals. Her scientific interests concern mostly andrology, molecular aspects of animal reproduction, use of biotechniques and avian reproductive biology. She was researcher in many scientific projects and the leader researcher of three projects financed by NCN. She was also a leader of the project titled *“Development of tools to create and analyze new extenders for semen cryopreservation”* realized with the cooperation of dr Pascal Froment (INRAE). She gained professional experience and practical skills during internships in Germany, Belgium, France and Spain and attending workshops and conferences. She participated in several courses on flow cytometry, molecular techniques and biotechniques. Agnieszka is an active member of Polish Society for Biology of Reproduction and Association for Applied Animal Andrology. She is an internationally recognized reviewer of such journals as e.g. *Animals*, *Animal Reproduction Science*, *Asian Journal of Andrology*, *Cryobiology*, *Reproduction in Domestic Animals*, *Theriogenology*. Since 2020 she is Editorial Board Member of *Animal Reproduction Science*, and Special Editor of Special Issue in *Animals* *“Current Status and Advances in Semen Preservation”*.

## Spermatozoal functions and characteristics - assessment tools in obesity

PARTYKA A, NAPIERKOWSKA S, NIŻAŃSKI W

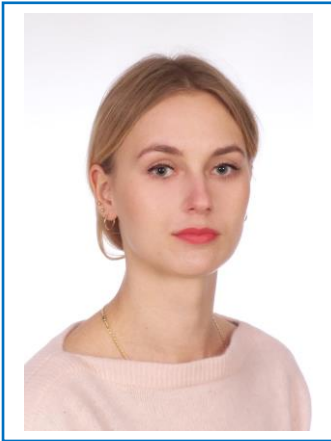
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**BACKGROUND:** Several studies have documented that obesity interferes with male reproductive function. To find possible obesity-mediated mechanisms that lead to male infertility, the assessment of conventional semen parameters (e.g., sperm concentration, motility, and morphology) is the most frequently performed. However, an in-depth examination of the biofunctional parameters of the sperm and also the molecular composition of the sperm is becoming more and more often chosen. The evaluation of non-conventional sperm parameters can provide important information that could not be obtained through classical seminal analysis alone and that, in turn, may be very useful to identify the causes that are responsible for the male infertility.

Many functional sperm tests using computer-assisted sperm analysis (CASA) and flow cytometry have become the gold standard for the evaluation of semen in specialized andrology laboratories. The markers that are worth paying attention are: integrity of the sperm plasma membrane evaluated with SYBR-14 and propidium iodide, mitochondrial activity evaluated by JC-1, sperm DNA integrity checked by acridine orange, or apoptosis evaluated by YO-PRO-1 or Annexin V. Furthermore, among the several mechanisms that are responsible for sperm damage in obese males, the state of oxidative stress plays a pivotal role. Oxidative stress can be assessed both using flow cytometry with fluorescent probe stained spermatozoa or by performing photometric or luminescent evaluations of seminal plasma.

Recently, imaging flow cytometry that combines the statistical power and fluorescence sensitivity of standard flow cytometry with the spatial resolution and quantitative morphology of digital microscopy has started to be used to analyze biomarkers in sperm cells.

**Keywords:** semen assessment, computer-assisted sperm analysis, flow cytometry, fluorescent stains



**WERONIKA BIERNAT, PhD**  
**UNIVERSITY OF AGRICULTURE IN KRAKOW, POLAND**

Weronika works as an assistant professor at the University of Agriculture in Krakow at the Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Science. Her scientific and research activity concerns the identification of processes accompanying the phenomenon of seasonality of reproduction in sheep, including the verification of hypotheses related to the seasonally reversible process of leptin resistance. In recent years, her research has focused on the role of two proteins: leptin and resistin - secreted by adipose tissue, in shaping the phenomenon of leptin insensitivity at the level of the central nervous system. In the research, she focused primarily on the molecular mechanism underlying leptin resistance resulting from the influence of resistin on the leptin signaling pathway and the involvement of the cytokine suppressor factor SOCS-3 protein in this process in the context of the length of the day - photoperiod and metabolic status of the female. She also participated as a co-worker in the implementation of the project *"The determination of factors affecting the transfer of leptin through blood-brain barrier (BBB) and the phenomenon of leptin resistance"*. During her short scientific career she published 7 peer-reviewed papers and 11 conference papers including poster and oral presentations.

## The roles of leptin and resistin in reproduction and leptin resistance in sheep

BIERNAT W, SZCZESNA M, KIRSZ K, ZIEBA DA

*Department of Animal Nutrition and Biotechnology, and Fisheries, Faculty of Animal Sciences,  
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**BACKGROUND:** Adipocyte-derived leptin and resistin are strongly involved as major peripheral signals that control body fat reserves and synthesis and release reproductive hormones. Seasonal breeding is a remarkable, adaptive feature that allows sheep to optimize their survival and reproductive success. Our group indicated for the first time that resistin is involved in the regulation of pituitary hormone secretion and that this effect is differentially mediated during the LD and SD seasons.

**MATERIALS & METHODS:** Experiments were conducted during both short (SD) and long day (LD) seasons. Thirty Polish Longwool ewes, a breed that exhibits a strong seasonal reproductive pattern, were ovariectomized with estrogen replacement using subcutaneously inserted estradiol implants. Treatments consisted of the intravenous injection of control (saline) or recombinant bovine resistin: control (Control; n = 10), R1 (1.0 µg/kg BW; n = 10), and R2 (10.0 µg/kg BW; n = 10).

**RESULTS:** Intravenous infusion of resistin increases the mean circulating concentrations of leptin and decreases luteinizing hormone in a dose-dependent manner during both the long-day and short-day seasons. Furthermore, exogenous resistin increased suppressor of cytokine signaling (SOCS)-3 mRNA expression only during the LD season, when the leptin resistance/insensitivity phenomenon was observed in the arcuate nucleus, preoptic area, and anterior pituitary.

**SUMMARY:** Both leptin and resistin operate at the level of the central nervous system to regulate reproductive processes, suggesting the activation of several common signaling pathways. The most recent experiments suggest that resistin may be another factor involved in central leptin resistance in animals, including seasonally breeding ewes.

**Keywords:** leptin, leptin resistance, resistin, sheep, photoperiod

**Funding:** supported by National Science Centre, Poland (2015/19/B/NZ9/01314)



**KAROLINA PICH, PhD STUDENT**  
**JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

Karolina is a PhD student at the Jagiellonian University in Cracow, Poland under the supervision of Professor Agnieszka Rak and co-supervision of Dr Patrycja Kurowska. Karolina is strongly interested in reproductive endocrinology focused on the impact of metabolic and environment factors on female reproductive function. In 2021 she received her MSc degree studying the effect of a mixture of persistent environmental pollutants on the viability of human ovarian granulosa cells - HGrC1 line.

Now she working on the expression and role of omentin-1 in the ovary of Large White and Meishan pigs, as a part of OPUS 19 project (2020/37 /B /NZ9 /01154) financed by the NCN: "*OMENTIN1 as a new regulator of female reproduction - expression and role in the pituitary - ovary axis. Study on swine model at different metabolic status*". This project is implemented in collaboration with Professor Joëlle Dupont's team from INRAE in France, where she realized an internship financed by the Society of Reproductive Biology. Karolina is the author of 5 publications, which concern the expression of adipokines in the ovary and placenta cells as well as their role in such processes like proliferation and apoptosis. She also actively participates in scientific conferences. Moreover, Karolina received funding for her study as part of the "Research Support Module" project as well as funding for participation in an international scientific conference by "Visibility & Mobility Module" project financed by the Jagiellonian University. Karolina is a member of the Polish Copernicus Society of Naturalists and the Society for Reproductive Biology.

## Effect of omentin on steroid synthesis in porcine ovarian follicles. *In vitro* study.

PICH K<sup>1</sup>, RESPEKTA N<sup>1</sup>, KUROWSKA P<sup>1</sup>, GUZMAN P<sup>1</sup>, SMOLIŃSKA N<sup>2</sup>, DUPONT J<sup>3</sup>, RAK A<sup>1</sup>

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**BACKGROUND:** The main function of the ovary is production of steroid hormones: progesterone (P<sub>4</sub>), testosterone (T) and estradiol (E<sub>2</sub>). Disturbances in their synthesis leads to numerous of reproductive pathology. Omentin is a newly discovered adipokine that regulates the body's energy metabolism; its expression has been demonstrated in ovary, while its effect on ovarian endocrine function still remain unknown. The aim of the study was to compare the dose-dependent effect of omentin on basal, as well as follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-induced secretion of steroid hormones (P<sub>4</sub>, E<sub>2</sub> and T) as well as expression of the steroidogenic pathways enzymes CYP11A1, CYP19A1, as well as StAR protein in two breeds of pigs - Large White (LW), with normal body weight and obese Meishan (MS).

**MATERIALS & METHODS:** Coculture of ovarian granulosa and theca cells (10-12 days of estrous cycle) were stimulated with omentin (10-100 ng/ml), FSH or LH (100 ng/ml), as well as omentin in combination with FSH or LH for 48h. The concentration of steroids in the culture medium and protein expression of enzymes was determined using ELISA or Western blot, respectively. Statistical analysis was performed using one-way ANOVA and Tukey's test.

**RESULTS:** We observed that omentin have a negative effect on basal P<sub>4</sub> and E<sub>2</sub> secretion in both breeds, as well as StAR and CYP11A1 protein expression. Additionally we noted that omentin increase protein expression of CYP19A1 in LW and MS pigs. Modulatory effect on LH and FSH stimulated steroidogenesis was observed in both breeds.

**SUMMARY:** Our study show that omentin is a novel regulator of endocrine function in the porcine ovary, and its effect depends on the omentin dose.

**Keywords:** omentin, pigs, ovary, steroidogenesis, metabolic status

**Funding:** supported by National Science Centre, Poland (OPUS19 2020/37/B/NZ9/01154). Karolina Pich was founded by finance from Doctoral Society at Jagiellonian University in Kraków.





**NATALIA RESPEKTA, PhD STUDENT  
JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

Natalia is a PhD student at Jagiellonian University in Krakow and working in Professor Agnieszka Rak's team. She received M.S. in Poland, where she studied the effect of vitamin C on ovarian human cancer cell lines. In her doctoral dissertation, the main field of her research is female reproduction at hypothalamus and pituitary levels, especially investigation of the omentin-1 expression and its effect on the porcine anterior pituitary cells functions proliferation, endocrinology as well as global transcriptome and proteome of two breeds of pigs: genetically fatty Meishan and Large White. Natalia completed a national internship at the University of Warmia and Mazury in Olsztyn, improving laboratory skills regarding primary *in vitro* cultures, and a foreign internship at the INRAE to enable studies on the pituitary gland of the Meishan breed. The results of her research have been presented at national and international conferences. Due to her involvement in experiments conducted by students and doctoral students, she is the author of the original paper and co-author of 7 research papers in peer-reviewed journals with a total impact factor of 31,189. Natalia improved their scientific workshop through participation in many methodological training courses and teaching classes. Natalia is a member of the Polish Copernicus Society of Naturalists and the Society for Reproductive Biology.

## Expression and action of omentin on porcine pituitary cell function

RESPEKTA N<sup>1</sup>, PICH K<sup>1</sup>, MLYCZYŃSKA E<sup>1</sup>, OPYDO M<sup>2</sup>, KISIELEWSKA K<sup>3</sup>, KOPIJ G<sup>3</sup>, DOBRZYN K<sup>4</sup>, ZAOBIDNA E<sup>3</sup>, DUPONT J<sup>5</sup>, SMOLIŃSKA N<sup>3</sup>, KAMIŃSKI T<sup>3</sup>, RAK A<sup>1</sup>

*<sup>1</sup>Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Poland; <sup>2</sup>Department of Animal Anatomy and Physiology, University of Warmia and Mazury in Olsztyn, Poland; <sup>3</sup>Department of Zoology, University of Warmia and Mazury in Olsztyn, Poland; <sup>4</sup>INRAE, Unité Physiologie de la Reproduction et des Comportements, France*

**BACKGROUND:** Omentin is an adipokine that is expressed in many tissues and organs, including visceral adipose tissue, heart, and ovarian cells, and impacts their functions on the cellular level. The aim of the study was to demonstrate the expression of omentin-1 in the porcine anterior pituitary cells (AP), and its impact on the AP cells proliferation and secretion of gonadotropins.

**MATERIALS & METHODS:** We investigated the expression of the omentin gene and protein by RT-qPCR, Western Blot, and immunofluorescence in AP in Large White pigs on days 2-3, 10-12, 14-16, and 17-19 of the estrous cycle. Next, AP cells were isolated from pigs to check the effect of omentin on the cell cycle, proliferation, expression of cyclin A, B, D, E, and marker of proliferation PCNA and secretion of FSH, LH. Statistical analysis was performed using the student's T-test, one-way ANOVA, and Tukey's post-hoc test.

**RESULTS:** The expression of omentin fluctuated during the cycle: mRNA increased from days 10-12 to 17-19, whereas protein concentration was highest on days 10-12 and lowest on days 17-19. Moreover, omentin stimulated proliferation and increased the percentage of cells in the G2 / M phase of the cell cycle but decreased cyclins and PCNA on mRNA levels. However, omentin increased LH secretion at concentrations of 10 and 50 ng/ml and showed a dose-dependent effect on the FSH release of the AP cells in pigs.

**SUMMARY:** For the first time, our data showed the expression of omentin in AP, and it suggests that omentin impacts proliferation, the cell cycle, and secretion of gonadotropins in porcine anterior pituitary cells.

**Keywords:** omentin, pituitary, pigs, proliferation, cell cycle, secretion, FSH, LH.

**Funding:** National Science Centre, Poland, OPUS19 2020/37/B/NZ9/01154. Natalia Respekta was funded by a grant from the Priority Research Area (U1U/W18/NO/09) under the Strategic Programme Excellence Initiative at Jagiellonian University.



**EWA MLYCZYŃSKA, PhD STUDENT**

**JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

Ph.D. student at Jagiellonian University in Kraków in Professor Agnieszka Rak's team. In 2019, she obtained a MSc in biology at the Faculty of Biology of the Jagiellonian University and continues her scientific adventure in the field of reproductive biology. The main subject of her research is the role of metabolic hormones belonging to the group of adipokines and neuropeptides in the regulation of reproductive functions. Her research as part of doctoral dissertation concerns the expression and role of visfatin and phoenixin-14 in the porcine corpus luteum. Ewa successfully obtained funds for her research and currently she is a PI of the PRELUDIUM 19 project (2020/37/N/NZ9/00981) financed by the NCN. In addition, she is a co-performer in OPUS 16 project (2016/22/M/NZ9/00316), carried out with the University of Warmia and Mazury in Olsztyn in a scientific consortium. In the past, she was also a co-performer in HARMONIA 7 project (2016/22/M/NZ9/00316) carried out with Professor Joelle Dupont from INRAE. Participation in research projects as well as involvement in the work of team made her a co-author of 22 scientific publications, including 3 with the first authorship, with a total impact factor of 91,592 as also 1 book chapter. She has presented her research at numerous conferences in Poland and abroad. In 2021, she received a scholarship from French Government to stay in a French research centre and thus she completed an internship in the laboratory of Professor Joelle Dupont which will be continued in the future. In addition, she had the opportunity to complete an internship at the Department of Animal Physiology of University of Warmia and Mazury in the laboratory of Professor Tadeusz Kamiński, where she could improve her methodological workshop. Moreover, Ewa is a member of several prominent societies including the European Society for Domestic Animal Reproduction, the Polish Copernicus Society of Naturalists, and the Society for Reproductive Biology.

## Visfatin in the porcine corpus luteum: expression and action on steroid synthesis and angiogenesis

MLYCZYŃSKA E<sup>1</sup>, ZAOBIDNA E<sup>2</sup>, RYTELEWSKA E<sup>2</sup>, RESPEKTA N<sup>1</sup>, DAWID M<sup>1</sup>, GUDELSKA M<sup>2</sup>, KOPIJ G<sup>2</sup>, DOBRZYŃ<sup>3</sup>, KIEŻUN M<sup>2</sup>, SMOLIŃSKA N<sup>2</sup>, KAMIŃSKI T<sup>2</sup>, RAK A<sup>1</sup>

<sup>1</sup>Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland; <sup>2</sup>Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland; <sup>3</sup>Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

**BACKGROUND:** Visfatin is one of the adipokines that creates a hormonal link between energy metabolism and female reproduction. Recent study documented visfatin expression in the ovary and action on follicular cells; however, expression of visfatin in luteal cells is still undiscovered. Therefore, the aim of this study was to investigate expression of visfatin in porcine corpus luteum (CL) and *in vitro* effect of visfatin on progesterone (P<sub>4</sub>) and angiogenic factors secretion during the estrous cycle.

**MATERIALS & METHODS:** CL were collected from gilts on days 2–3, 10–12, 14–16 of the estrous cycle for the study of visfatin transcript and protein expression as well for visfatin action on steroidogenesis and angiogenesis. Luteal cells were stimulated with visfatin (1, 10, 100 ng/ml) and next P<sub>4</sub>, VEGFA, FGF2, ANG2 and iNOS concentrations were checked. Additionally, we investigate the expression of proteins involved in P<sub>4</sub> synthesis (StAR, CYP11A1 and 3βHSD) and activation of MAPK, AMPKα and AKT kinases. Moreover, involvement of these kinases and insulin receptor in visfatin action were determined using pharmacological blockers of signaling pathways.

**RESULTS:** The study demonstrated variable expression patterns of visfatin in CL between transcript and protein levels, dependent of days of estrous cycle. Visfatin modulates secretion of P<sub>4</sub> and angiogenic factors depending on visfatin doses and stage of luteal phase. A stimulatory effect of visfatin on the protein expression of StAR, CYP11A1 and 3βHSD and activation of studied kinases was noted. Moreover, insulin receptor was involved in visfatin action on P<sub>4</sub>, ANG2 and VEGFA secretion. MAPK, Akt and AMPKα were involved in visfatin action on P<sub>4</sub> secretion, while MAPK and Akt for ANG2 and MAPK for VEGFA.

**SUMMARY:** Our study proved that visfatin is expressed in luteal cells and changing during the estrous cycle in pigs. Moreover, it is a novel regulator of steroidogenesis and angiogenesis in luteal cells.

**Keywords:** visfatin, corpus luteum, pig, steroidogenesis, angiogenesis

**Funding:** Supported by National Science Centre, Poland (OPUS16 2018/31/B/NZ9/00781).



**DOMINIKA WACHOWSKA, PhD STUDENT  
JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

1<sup>st</sup> year PhD student at Jagiellonian University in Cracow, Poland. She graduated with a MSc in Biology (spec. Molecular Biology). From the beginning of 3<sup>rd</sup> year of studies, Dominika was involved in a project of Prof. Paweł Grzmil in the Laboratory of Genetics and Evolutionism regarding genetic background of spermatogenesis and male infertility, where she found molecular mechanisms of fertility regulation. Dominika got a scholarship from Erasmus+ exchange program, for one semester of studies in France at the University of Montpellier. She spend six-month internship in the team of Dr Solange Desagher '*Control of neuronal cell death*' at the Institute of Molecular Genetics in Montpellier, CNRS. Indeed, in this team, she conducted a project concerning the role of alpha-synuclein in cytochrome c release during neuronal apoptosis. Dominika start her PhD thesis as a part of NCN project SONATA BIS "*Expression and role of asprosin/OLFR734 in the regulation of mouse hypothalamus and pituitary cells function*" in Prof. Agnieszka Rak's team.

## Asprosin – new adipokine in female reproduction

WACHOWSKA D, RAK A

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

**BACKGROUND:** Asprosin with its receptor OLF734 is recently discovered fasting-induced glucogenic adipokine. This hormone is mainly synthesized in white adipose tissue and the serum asprosin concentration is higher in obese women and mice. Nowadays, obesity has become a global epidemic which is linked to various co-occurring conditions, such as metabolic syndrome, type 2 diabetes, cancer, stroke and infertility. It is proved that asprosin affects male fertility. However, there is no data of the effects of asprosin on female reproduction functions. Our new project hypothesis assumes that asprosin is involved in the regulation of energy metabolism and plays role as new marker of female fertility. To investigate the potential link involved in the regulation of reproduction and animal metabolic status, the expression and the role of asprosin on female reproduction at the level of the ovaries and the upstream branches of the hypothalamus-pituitary-ovarian (HPO) axis will be verified.

**MATERIALS & METHODS:** Experiments will be conducted on C57BL/6J female mice lean (RCD – regular chow diet) vs obese (HFD - high fat diet). *In vitro* study concerns using both, hypothalamic GT1-7 and pituitary L $\beta$ T2 cell lines. During next step of this project, human granulosa cells (Gc), plasma and follicular fluid (FF) collected from lean and obese women will be analyzed and asprosin knockout (KO) mice will be added for the analyses. For verifying the expression and levels of asprosin/OLF734 mRNA and protein and the cellular colocalization, we plan to use: RT-qPCR, Western blot, ELISA, *in situ* hybridization, gene silencing (siRNA), the proliferation and apoptotic enzyme tests and proteomic and bioinformatic analyses.

**RESULTS:** The expected results will provide new, extremely valuable information on the molecular control (transcriptome/proteome/functioning) of female reproduction under different metabolic conditions. The data from this 5-year project will have clinical benefits, identification of the role of asprosin on female reproduction upstream structures of HPO axis could put as a step forward to new prevention and treatment strategies in female infertility as well obesity which now is widespread worldwide.

**SUMMARY:** Asprosin is potential new marker of female reproductive functions.

**Keywords:** Asprosin, obesity, HPO axis, induced-obesity mice

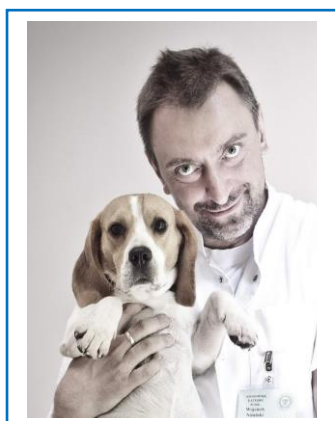
**Funding:** SONATA BIS 11 (2021/42/E/NZ4/00088) funded by NCN. Dominika Wachowska was funded by finance from Doctoral Society at Jagiellonian University in Kraków.



## SECOND PANEL

*The role of adipokines on fertility regulation at peripheral levels: ovary & testis*

**Moderators:** Wojciech Niżański & Pascal Froment



**PROFESSOR WOJCIECH NIŻAŃSKI**  
**WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES,**  
**POLAND**

Wojciech was graduated in veterinary medicine in year 1992. The doctoral thesis entitled: *“Investigation on the semen preservation in low temperature”* he defended in 1999 and habilitation concerning *“The relationship between the effectiveness of the artificial insemination and in vitro semen characteristics in dog”* he defended in 2009. From 2009 onwards he became the head of the Department of Reproduction and Clinic of Farm Animals, at Faculty of Veterinary Medicine in Wroclaw University of Environmental and Life Sciences. He obtained a title of professor in 2014. Since 2019 he is Chairperson of the Discipline Council for Veterinary Medicine in Wroclaw. Wojciech is an active clinician mostly working on artificial insemination, reproductive disorders in males and females, obstetrics, neonatology and assisted reproductive techniques. He is also a researcher on the field of the use of Assisted Reproduction Technologies in programs of rescue of endangered animal species. He established in Wroclaw Bank of Gametes and Somatic Cells of Companion Animals and Endangered Animal Species. He published over 200 papers intended for scientists and practicing veterinarians. He was the leader in 12 research projects financed from the external sources and the investigator in many others. He is an active member in international organisations, e.g. of European Veterinary Society for Small Animal Reproduction (EVSSAR), where he was a former President; Board Member and Editor and he is a current EVSSAR Representative in Poland. He is also a member in Polish Society for Biology of Reproduction (Member of the Board), Polish Society of Veterinary Science, PSAVA President Elect and Chairperson of Committee of Veterinary Sciences and Reproductive Biology, Polish Academy of Sciences. He is a valued speaker and workshops’ master during many congresses worldwide (e.g. in Budapest, Estoril, Milan, Berlin, Hanover, Moscow, Oslo, Cordoba, Ghent, Whistler), as well as during various conferences in the country.



**FROMENT PASCAL, PhD**

**Director of Research**

**NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE, FOOD AND THE ENVIRONMENT, FRANCE**

Pascal is a Director of Research in metabolism and fertility in INRAE Institute, France. He received his M.S., Ph.D. in 2003, and HDR in 2009. His research during the past 15 years has been focused on reproductive physiology and the relationship between energy metabolism and reproductive functions in avian and mammals models. He has an expertise and a leadership in the action of insulin sensitivity drug in the testis function including PPAR and AMPK sensor protein. He has developed different strategy inactivating cellular pathways involved the energy metabolism in Leydig and Sertoli cells. He has constructed a network in France with academic, lab, Hospital services, and with international collaborators (Dr. C.Giulivi and D. Rappollee, USA; Dr. R. Gilchrist, Australia; Dr. A. Partyka, Poland, DrJ.C. Marine, Belgium) and produced over 50 peer-reviewed publications, and presented results in over 100 poster/oral communications in national and international meeting. He is a PI and work-package leader of 7 projects in the last 10 years, and to better decipher the role of energy in the gonad, both *in-vivo* and *in vitro*. He give lectures for graduate students at the University of Paris (Master "Reproduction and development") and at the University of Tours (Master „Reproduction”).

## ORAL PRESENTATION II



PROFESSOR CECILIA DALL'AGLIO,  
UNIVERSITY OF PERUGIA, ITALY

Cecilia is a Professor of Veterinary Histology and Anatomy, at the Department of Veterinary Medicine, in Perugia, Italy. She is the Coordinator of the degree course in Veterinary Medicine, University of Perugia, starting from the academic year 2021/2022. For several years, her scientific activity has been dedicated to the study of molecules functionally linked to the control of appetite and metabolism. Among these, in particular, the adipokines have been studied to highlight their presence and distribution in the digestive and reproductive systems of several animal species. In recent years she has dedicated her research activity to the study of the effects of climate and nutrition on the expression of adipokines, intended as an indicator of animal welfare. Below, some of her last participations to projects: -Scientific coordinator of the project entitled "*Study of apelin and resistin adipokines in the female genital tract of sheep reared on pastures at different nutritional levels*" (Fondo d'Ateneo per la Ricerca di Base. year 2015) -Scientific coordinator of the project entitled "*Factors for inducing ovulation in seminal plasma: innovative strategies to increase reproductive efficiency in pets*" (Fondazione Cassa di Risparmio di Perugia, Ricerca Scientifica e Tecnologica. year 2018) -Participant in the project "*Preliminary study on the possible role of the endocannabinoid system and phytocannabinoids in the modulation of canine reproductive system neoplasms: expression of cannabinoid receptors in the healthy reproductive system and with neoplasms, and the pharmacokinetics of cannabidiol*" (Fondo d'Ateneo per la Ricerca di Base. year 2019). She is responsible of an international collaboration agreement within the PhD course in "*Health and Veterinary experimental sciences*" between the University of Perugia and the University of Veterinary Medicine and Pharmacy of Košice, Slovakia. She is a member of the Italian Association of Veterinary Morphologists (AMV) and of the Italian Society of Veterinary Sciences and a component of the "Animals" editorial board. She co-authored more than 80 papers.

## Adipokines expression in ewes subjected to the influence of different nutritional level

DALL'AGLIO C<sup>1</sup>, SCOCCO P<sup>2</sup>, MERCATI F<sup>1</sup>

<sup>1</sup>Department of Veterinary Medicine, University of Perugia; <sup>2</sup>School of Biosciences and Veterinary Medicine, University of Camerino

**BACKGROUND:** The environmental characteristics combined with the food availability condition the life and well-being of farmed animals: whereas well-being is responsible for productivity, it is important to find the right conditions also in the perspective of an economic return for the farmer. The aim of our research was to evaluate adipokines as possible markers of animal welfare investigating these molecules in peripheral organs of animals subjected to different diets and environment conditions.

**MATERIALS & METHODS:** 15 sheep were free to graze on a pasture of Central Apennine, from June to the pasture maximum flowering (MxF group). From pasture maximum flowering, 10 ewes were divided into 2 groups: the MxD (maximum dryness) group was free to graze on pasture while the EXP (experimental) group was also supplemented. The adipokine apelin together with its receptor was investigated in the mammary glands, genital system and digestive tract.

**RESULTS:** Apelinergic system was observed in all organs evaluated. The comparison between groups of sheep suggested a role of the molecule in the local functional regulation of the organs evaluated. Correlation with nutrition was clearly observed in the digestive tract.

**SUMMARY:** Our study aimed to investigate if adipokines show variations due to diet and environment. The organs and systems studied are important for farm animal productivity. We hypothesized that the apelinergic system can play a role in regulating the local function of the digestive tract, mammary gland and female genital system. Correlating the molecules with the functional moments, nutrition and the environment is important for properly managing animals and promoting their productivity.

**Keywords:** apelin; immunohistochemistry, RT-PCR; ovine; grazing

**Funding:** This research was funded by the grant "FAR - CLIMAPP 2014-2015", assigned to Prof. Andrea Catorci by the University of Camerino, and the grant "Basic Research 2015, University of Perugia" assigned to the project "Study of apelin and resistin in the genital tract of sheep reared on pastures with different nutritional value", scientific coordinator Prof. Cecilia Dall'Aglio.



**PATRYCJA KUROWSKA, PhD**  
**JAGIELLONIAN UNIVERSITY IN KRAKÓW, POLAND**

Patrycja graduated biology at Jagiellonian University in 2017 with a specialization in genetics and biology of reproduction. In the same year she started her doctoral studies under the supervision of Professor Agnieszka Rak. Since then, her research interests have been related to understanding the relationship between the body's energy metabolism and female reproduction. Patrycja defended her doctoral dissertation "*Expression and role of vaspin in porcine ovarian cells*" in 2021. During her doctoral studies, she was involved in Professor Rak's projects incl. the NCN HARMONIA carried out in cooperation with Professor Joelle Dupont. Patrycja was PI in 2 projects from NCN: PRELUDIUM and ETIUDA, which concerned learning the role of adipokines in the regulation of ovarian physiology. She improved her methodical workshop, among others the methods of *in vitro* maturation of oocytes, human granulosa cell culture and genes silencing in porcine ovaries thanks to numerous visits in the Professor Dupont laboratory at INRAE, due to support of Society of Reproductive Biology and France Government. After defending PhD she completed a research internship in the Laboratory of Reproductive Biology at the Kyoto University in Japan under the supervision of Professor Naojiro Minami, where she mastered the CRISPR/Cas9 gene editing method in fertilized embryo. Currently, dr Kurowska is professionally connected with the Jagiellonian University, where she is continuing her research as research assistant in Laboratory of Physiology and Toxicology of Reproduction. Since her doctoral studies, she have been a member of the Society for the Study of Reproduction (SSR), Polish Society of Reproduction and Society of Naturalists. Dr Kurowska is an author of 25 papers and 3 popular-science articles and her H-index is 10. She also presented her results 54 times on conferences including ESDAR, SSR and Society of Reproductive Biology. For her research activity Patrycja have been awarded by the Rector of the Jagiellonian University, Society of Reproductive Biology, as well as by Minister of Education and Science of Poland for Young Prominent Scientists.



## Expression and role of spexin in human granulosa cells

KUROWSKA P<sup>1</sup>, RESPEKTA N<sup>1</sup>, DAWID M<sup>1</sup>, SERRA L<sup>2</sup>, ESTIENNE A<sup>2</sup>, DUPONT J<sup>2</sup>, RAK A<sup>1</sup>

<sup>1</sup> *Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Krakow, Poland* <sup>2</sup> *Unité Physiologie de la Reproduction et des Comportements, INRAE, Nouzilly, France*

**BACKGROUND:** Spexin (SPX) is a neuropeptide expressed in the neurons of the hypothalamus that regulates energy homeostasis and reproduction. The aims of the research were determine the expression level of SPX and its galanin receptors GALR2 and GALR3 in human ovarian cells collected from women of polycystic ovarian syndrome (PCOS), as well as its role in proliferation, kinases pathways activation and steroidogenesis.

**MATERIALS & METHODS:** Follicular fluid and granulosa cells (Gc) were obtained from healthy women and diagnosed with PCOS (normal weight and obese). Next using human Gc and human granulosa-like tumor cell line KGN culture the effects of SPX on cell proliferation and PCNA level, kinases pathways activation and steroidogenesis measured by steroids secretion (P<sub>4</sub>, T and E<sub>2</sub>) and mRNA expression of StAR and enzymes: CYP11A1, HSD3B, CYP17A1, CYP19A1 were determined. Statistical analyses were performed by Graph Pad Prism 5.

**RESULTS:** The SPX concentration in the follicular fluid and its mRNA expression in Gc decreased in obese and PCOS women, while the expression of the GALR3 receptor was increased; SPX, GALR2 and GALR3 were localized in Gc. SPX reduced the proliferation and kinases AKT, PKA and STAT3 phosphorylation, and opposite effect was observed for ERK1/2. Additionally, SPX inhibited steroidogenesis: in KGN decrease T and E<sub>2</sub> secretion, as well as StAR, CYP11A1 and CYP17A1 expression, while in human similar effect observed for P<sub>4</sub> and E<sub>2</sub> (P<0.05).

**SUMMARY:** The obtained data clearly indicate that SPX expression in human ovary is dependent on metabolic and hormonal condition and its negative effect on ovarian physiology may be a medical target to restore fertility in PCOS suffering women.

**Keywords:** spexin, ovary, polycystic ovarian syndrome, obesity, proliferation, steroidogenesis, kinases activation

**Funding:** The research has been supported by a grant from the Priority Research Area (B.1.7.2021) under the Strategic Programme Excellence Initiative at Jagiellonian Univeristy. Patrycja Kurowska obtained a funding for conference participation costs from Foundation of Students and Alumni of JU "Bratniak".



## Chemerin produced locally within the reproductive tract impairs testicular function in roosters

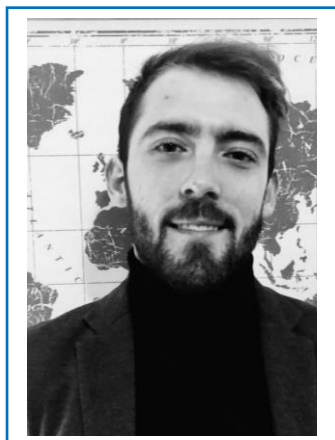
ESTIENNE A<sup>1</sup>, REVERCHON M<sup>2</sup>, PARTYKA A<sup>3</sup>, BOURDON G<sup>1</sup>, GRANDHAYE J<sup>1</sup>, BARBE A<sup>1</sup>, CALDAS-SILVEIRA E<sup>1</sup>, RAME C<sup>1</sup>, NIŻAŃSKI W<sup>3</sup>, FROMENT P<sup>1</sup>, DUPONT J<sup>1</sup>

<sup>1</sup>INRA UMR85 Physiologie de la Reproduction et des Comportements, France CNRS UMR7247 Physiologie de la Reproduction et des Comportements, France Université François Rabelais de Tours F-37041 Tours, IFCE F-37380 Nouzilly, France; <sup>2</sup>SYSAAF-Syndicat des Sélectionneurs Avicoles et Aquacoles Français, Centre INRA Val de Loire, F-37380 Nouzilly, France; <sup>3</sup>Department of Reproduction and Clinic of Farm Animals, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

Chemerin is an adipokine that plays a role in several biological processes such as adipogenesis and metabolism. Recent findings showed a negative correlation between plasma chemerin and female fertility parameters in chicken. So, we investigated the effects of chicken recombinant chemerin (ReCChem) on rooster sperm since the fertility of these animals presents a agronomic interest. We have shown that chemerin and its receptors CMKLR1, CCRL2 and GPR1 were expressed within the testis from the embryo stages to adulthood, with a lowest expression in adult testis. We also observed that chemerin levels were higher in blood than in seminal plasma. Qualitative analysis of roosters sperm highlighted a negative correlation between seminal plasma chemerin levels and the percentage of motility, progressive motility and the spermatozoa concentration. Furthermore, the percentage of static spermatozoa was correlated with chemerin concentration in seminal plasma. After *in vitro* experiments of rooster sperm treated with ReCChem and/or with an anti-CMKLR1 antibody, we showed that ReCChem inhibited sperm massal motility and this effect was abolished when sperm was pre-incubated with the anti-CMKLR1 antibody. Fresh rooster sperm was incubated with ReCChem and used for artificial insemination (AI). We observed a negative effect of chemerin on eggs fertility for the three first days after AI. Then eggs' fertility became identical between the tested conditions, suggesting a transitory negative effect of chemerin on sperm. Taken together, seminal chemerin levels are negatively associated to the rooster fertility and chemerin produced locally by testis or male tract could negatively affect sperm quality through CMKLR1.

**Keywords:** rooster, chemerin, sperm, steroidogenesis, fertility

**Funding:** This project was supported by Région Centre Val de Loire (PREVADI grant number 32000820).



**GUILLAUME BOURDON, PhD STUDENT  
NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE,  
FOOD AND THE ENVIRONMENT, FRANCE**



Guillaume, a PhD Student in the SENSOR Laboratory at INRAE in Tours in France. During his thesis, under the supervision of Professor Pierre-Henri Ducluzeau and the researcher Pascal Froment, he studies the effects of a hepatokine Fibroblast Growth Factor 21 (FGF21) on reproduction. He works on human and mouse reproductive projects, in the goal to highlight the impact of metabolism on sperm and steroidogenesis.

## The hepatokine FGF21 increases the human spermatozoa motility

BOURDON G<sup>1</sup>, ESTIENNE A<sup>1</sup>, CHEVALEYRE C<sup>1</sup>, RAME C<sup>1</sup>, GUERIF F<sup>2</sup>, BRUN J-S<sup>3</sup>, VASSEUR C<sup>3</sup>, FROMONT G<sup>4</sup>, INGRID P<sup>5</sup>, DUFOUR D<sup>6</sup>, ERIKA C-S<sup>1</sup>, DUPONT J<sup>1</sup>, FROMENT P<sup>1</sup>, DUCLUZEAU P-H<sup>1</sup>

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**BACKGROUND:** Lifestyle, environment and excess bodyweight are not only associated with an increased risk of metabolic disorders, such as type 2 diabetes, but also to others pathological processes, such as infertility. A hormone produced mainly by the liver, called fibroblast growth factor 21 (FGF21), is closely linked to the energy status and present high blood levels in patients suffering from obesity or insulin resistance. Recently, the FGF21 pathway has been shown to be associated with female fertility disorders, however, few data about the role of FGF21 in human male fertility has been described.

**MATERIALS & METHODS:** In collaboration with Tours Hospital and Vinci Clinic, we analyzed the FGF21 levels in blood and seminal fluid from patients, and performed immunochemistry/Western-Blot on human testis samples. Finally, on fresh human spermatozoa we studied the impact of FGF21 on motility, ATP production and oxidative stress.

**RESULTS:** In human testis, we showed the expression of FGF21 receptors, FGFR1/3/4, as well as cofactor KLB, mainly in Leydig and germ cells. In spermatozoa, FGFR1/3 are co-localized with KLB in the intermediate piece, site of localization of the mitochondria. Treatment of fresh semen with increasing concentration of FGF21 showed a dose-dependent enhancement in sperm mobility and in mitochondrial activity.

**SUMMARY:** Overall, we measured FGF21 in seminal fluid and highlighted a potential local production of FGF21 in male reproductive tract tissues. We observed the ability of FGF21 to improve sperm motility and quality of the human spermatozoa parameters. We propose that FGF21 is a metabolic factor involved in the crosstalk between human metabolism and spermatogenesis.

**Keywords:** FGF21, FGFR1, KLB, FGFR3, Spermatozoa, Motility

**Funding:** This work was funded by INRAE and « Région Centre Val de Loire » / Collaboration with CHRU of Tours & Vinci Clinical Institute of Tours



**MATHILDE DAUDON, PhD STUDENT  
NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE, FOOD AND THE  
ENVIRONMENT, FRANCE**

Mathilde is doing her PhD thesis in a French-Quebecois cotutelle with Doctor Christopher Price from the faculty of veterinary medicine at Saint-Hyacinthe (Canada) and Doctor Joëlle Dupont from INRAE at Nouzilly (France). Mathilde carried out her master in reproductive biology in the University of Tours (France) and performed her Master's internship in Canada. She is working during her PhD on Fibronectin type III domain-containing (FNDC) and their impact on energy metabolism and ovarian functions in dairy cows. The aim of her project is to find new diagnostic biomarkers of follicle quality according to metabolic disorders.

## Fibronectin Type III domain containing 5 (FNDC5) expression in bovine ovary and *in vitro* effect on bovine granulosa cell proliferation and steroidogenesis

DAUDON M<sup>1,2</sup>, RAME C<sup>1</sup>, PRICE C<sup>2\*</sup>, DUPONT J<sup>1\*</sup>

<sup>1</sup> CNRS, IFCE, INRAE, Université de Tours, Nouzilly, France; <sup>2</sup>Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Canada; \*co-last author

**BACKGROUND:** In worldwide, the fertility of dairy cows has declined significantly since the 1950s. It's widely accepted that at the beginning of lactation the dairy cow is in negative energy balance (NEB) that decreases reproductive performance, and she therefore mobilizes adipose tissue for energy and milk production. Adipose tissue is a source of hormones (adipokines) and rapid adipose mobilization changes the circulating adipokine landscape. A recently characterized family of adipokine-myokines is the Fibronectin Type III domain containing (FNDC) proteins. The most studied is FNDC5 and its cleaved and secreted fragment called irisin. The effect of FNDC5 and irisin on metabolism during the lactation and on the functioning of the ovary in cows remains unknown. We hypothesized that FNDC5 and irisin are linked to NEB in dairy cows and could affect fertility by acting on ovarian steroidogenesis.

**MATERIALS & METHODS:** Thus, here we investigated firstly the expression of FNDC5 in bovine ovary, the effect of various adipokines on granulosa cell (GC) FNDC5 expression and the role of human recombinant irisin on GC proliferation and steroidogenesis *in vitro*.

**RESULTS:** We showed that FNDC5 was expressed in bovine ovary, and we detected FNDC5 in follicular fluid mostly in medium follicles as compared to small and large ones (p-value: 0.0002). By RT-qPCR we showed that visfatin increases *FNDC5* expression (p-value: 0.0152). Addition of human recombinant irisin to cultured GC increased cell proliferation in a dose dependent manner at 1, 10 and 100 ng/ml (p<0.001). Moreover, irisin decreased progesterone and oestradiol secretion at basal state and in response to FSH and IGF1 (p<0.0001).

**SUMMARY:** Taken together, these data show that FNDC5 is expressed in bovine ovary and modulates granulosa cell steroidogenesis.

**Keywords:** FNDC5, irisin, bovine, granulosa cells, fertility

**Funding:** This work was financially supported by INRAE and RQR (Réseau Québécois en Reproduction).

## THIRDPANEL

*The role of adipokines on fertility regulation at peripheral: uterus, placenta,  
embryo development & pregnancy*

**Moderators:** Nina Smolińska & Joelle Dupont



**PROFESSOR NINA SMOLIŃSKA,  
UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND**

Nina is a professor of natural sciences at the Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. In 2019, she was deputy dean for science of this Faculty. Currently, she is the chairwoman of the Scientific Council of the Biological Sciences Discipline and the Council of Scientific Excellence at the University as well as the member of the Scientific Council of the Doctoral School. She joined international labs such as Sanford-Burnham Medical Research Institute, USA and CiMUS, University of Santiago de Compostela, Spain. Her research concerns the hormonal regulation of mammalian reproductive processes. She deals with the reproductive endocrinology, especially the role of adipokines and orexins in the regulation of the reproductive system. She defined the expression and role of adipokines and orexins in the regulation of processes occurring in the hypothalamic-pituitary-ovarian axis (HPO), uterus and trophoblasts of domestic pigs during early pregnancy (period critical for embryo survival) and during the oestrous cycle as well as the expression and role of orexins and their receptors in the structures of the HPO and hypothalamic-pituitary-adrenal (HPA) axes of the European beaver depending on sex and the season of the reproductive activity. She identified the transcriptomic (global gene expression, alternative splicing, long non-coding RNAs and single nucleotide polymorphism) as well as proteomic changes in the pituitary, ovaries and uterus of domestic pig under the influence of adipokines. She published approx. 80 papers and over 110 abstracts cited 1,067 times with an h-index of 20. Professor Smolińska was the PI research supervisor of seven projects and a main investigator in seven other projects (five of them are being implemented). She supervised 6 PhD theses and about 30 engineer/bachelor's theses. In 2021, Prof. Smolińska received Silver Cross of Merit.



## ORAL PRESENTATION III



**MARTA KIEZUN, PhD**

**UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND**

Marta is an Assistant Professor at the Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn. She is a member of the Polish Society for Reproductive Biology and the Polish Physiological Society. Marta completed several short-term internships, i.a. at the University of Turku, Finland and Karolinska Institute in Stockholm, Sweden, and a 3-month internship at the University of Santiago de Compostela, Spain. Since 2019 she has been a member of the Scientific Council in the Discipline of Biological Sciences at the University of Warmia and Mazury in Olsztyn. Marta is a member of the research team investigating the role of various adipokines and neurohormones in the functioning of the hypothalamic-pituitary-ovary axis and uterus of the domestic pig. She defended her doctoral dissertation concerning the role of adiponectin in the regulation of porcine pituitary functioning in 2015. She participated in 2 scientific projects supported by the Ministry of Science and Higher Education and 9 grants supported by NCN of Poland. Marta was also a PI in a single research task Miniatura-1 supported by NCN. She participated in many international conferences, including International Congress on Pig Reproduction, the European Society for Domestic Animal Reproduction Congress and Animal Physiology. Marta is a co-author of 57 scientific papers. Her papers have been cited 639 times and her h-index is 15. In 2017 she was awarded the Scholarship of the Minister of Science and Higher Education for Young Prominent Scientists. She supervised 3 engineer and 2 master theses. Currently, she is a PI in the Sonata-16 project that aimed to evaluate the influence of omentin on the functioning of the porcine uterus during the periimplantation period.

## Adipokines, rising stars in early pregnancy theatre

KIEZUN M<sup>1</sup>, DOBRZYN K<sup>1,2</sup>, KAMINSKA B<sup>1</sup>, ZAOBIDNA E<sup>1,3</sup>, RYTELEWSKA E<sup>1</sup>, KISIELEWSKA K<sup>1</sup>, GUDELSKA M<sup>1</sup>, ORZECZOWSKA K<sup>1</sup>, KOPIJ G<sup>1</sup>, SZYMANSKA K<sup>1</sup>, ZARZECKA B<sup>1</sup>, BIAGINI A<sup>1</sup>, KAMINSKI T<sup>1</sup>, SMOLINSKA N<sup>1</sup>

<sup>1</sup>Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland; <sup>2</sup>Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland; <sup>3</sup>Department of Biochemistry, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland

**BACKGROUND:** Reproduction is one of the most complex and energetically demanding processes for the female organism. We assumed that adipokines, hormones produced mainly by the white adipose and involved in the regulation of metabolism, are a crucial link between energy balance and uterine functioning. For over two decades our research focuses on the presence, regulation of expression and physiological functions of various adipokines in the porcine endometrium during the oestrous cycle and early pregnancy.

**MATERIALS & METHODS:** Our research model are cyclic and early pregnant gilts. In our studies, we apply various molecular methods, including qPCR, Western blotting, RIA, ELISA or *in vitro* cell and tissue cultures, as well as high-throughput methods, like Microarrays, NGS and LC-MS.

**RESULTS:** We confirmed that several adipokines and their receptors are expressed in the porcine uterus, and their expression may be dependent on the physiological status of the animal, among others, the concentration of steroids and prostaglandins. We demonstrated that adipokines may affect functions of the endometrium that are pivotal for embryo implantation and pregnancy maintenance, such as steroidogenesis, prostaglandin and cytokine production and signalling, angiogenesis, cell-to-cell adhesion, as well as cell proliferation and apoptosis. Our studies provide also evidence that adipokines may affect the global transcriptome and proteome of endometrial cells.

**SUMMARY:** Although adipokines' functions in the regulation of metabolism seem to be well described, their role as a molecular link integrating metabolism and endometrial functions requires further in-depth exploration. The presented results suggest their potential importance as a target for reproduction management.

**Keywords:** adipokines, oestrous cycle, early pregnancy, reproduction, female, pig, implantation

**Funding:** This research was supported by the Polish State Committee for Scientific Research (projects: PBZ KBN-084/P06/2002JP, 0206.911, 0206.0805), Polish National Science Centre (Opus-2 2011/03/B/NZ9/04187, Opus-13 2017/25/B/NZ9/00040, Sonata-16 UMO-2020/39/D/NZ9/01009, Miniatura-1 2017/01/X/NZ9/00484, Miniatura-3 2019/03/x/nz9/00213, Preludium-16 2018/31/N/NZ9/00783, Preludium-20 2021/41/N/NZ9/02325)



**MONIKA DAWID, PhD STUDENT**  
**JAGIELLONIAN UNIVERSITY IN KRAKOW, POLAND**

PhD student at the Faculty of Biology at the Jagiellonian University. She carried out her bachelor's and master's thesis in Professor Agnieszka Rak' teams, where she was studied the influence of adipokines in the human placenta cells function. The subject of her PhD dissertation is to investigate the expression, immunolocalization and role of visfatin in the course of placental proliferation, apoptosis and endocrinology. Until now Monika carried out research within four scientific projects: she is the PI of Ministry of Education and Sciences (Diamond Grant IX) and a contractor in the NCN (HARMONIA, OPUS16) and UJ-POB (BiOS) projects, which concern the study of adipokines and neuropeptides effect in reproductive processes. During her master's studies, she was awarded a Minister's scholarship for significant achievements for the 2020/2021, and during her doctoral studies, received a French Government scholarship 2022 for a two-month research internship. Additionally, as part of the Excellence Initiative - Jagiellonian University, she became the laureate of the "Excellence Module" for a publication achievement and received funding under the "Visibility & Mobility Module" for participation in a foreign scientific conference as well as due to "Research Support Module" became a PI of mini grant which expands her PhD studies. In addition, she presented her research at national and international conferences and is the author of 14 scientific publications on JCR list as well as co-author of 2 popular science articles. In spare time, the student engages in popularizing science by participating in the organization of workshops at the Faculty of Biology, JU.

## Visfatin in human placenta cells

DAWID M<sup>1</sup>, GIERAS W<sup>1</sup>, OPYDO M<sup>2</sup>, RAK A<sup>1</sup>

<sup>1</sup> *Laboratory of Physiology and Toxicology of Reproduction; <sup>2</sup> Laboratory of Experimental Hematology  
Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow*

**BACKGROUND:** Expression and role of visfatin have been described in many organs, where regulates energy metabolism, insulin resistance and inflammatory responses. Its level changes during pregnancy, and in obese women as well as those diagnosed with some pregnancy pathologies it significantly increases. We indicated expression as well as immunolocalization of visfatin in human placental cells. The aim of this study was to determine the effect of: estradiol (E<sub>2</sub>), progesterone (P<sub>4</sub>), human chorionic gonadotropin (hCG) and insulin on the visfatin level as well as to examine the visfatin effect on the proliferation and cell cycle of JEG-3 trophoblasts.

**MATERIALS & METHODS:** JEG-3 were grown in DMEM/F12 with 1% FBS; to study the regulation of the visfatin level (Western blot, ELISA), cells were stimulate with different doses of: E<sub>2</sub>, P<sub>4</sub>, hCG and insulin for 24, 48, 72 h. In the second part of studies, cells were treated with visfatin or combined with visfatin and insulin to explore the effect on proliferation (alamarBlue assay), cell cycle (flow cytometry) and the level of cyclins D, E, A, B and the PCNA (real time PCR, Western blot). We also investigated the involvement of ERK 1/2, AKT, STAT3 and AMPK $\alpha$  kinase pathways in placenta cells proliferation. Statistical analysis were performed in Graph Pad Prism 8 software, using one-way ANOVA and Tukey's test.

**RESULTS:** We demonstrated that E<sub>2</sub>, P<sub>4</sub>, hCG and insulin significantly stimulated visfatin level in JEG-3. It was investigated that visfatin alone and visfatin together with insulin inhibit significantly the proliferation of JEG-3. Moreover, we demonstrated that ERK 1/2, STAT3 and AMPK $\alpha$  signaling pathways are involved in the anti-proliferative activity of visfatin in placenta cells. Visfatin decreased the course of the cell cycle in the G2/M phase as well as the level of PCNA and all cyclins.

**SUMMARY:** Levels of visfatin in placenta cells are dependent by E<sub>2</sub>, P<sub>4</sub>, hCG and insulin, which can explain by changes plasma levels of visfatin during pregnancy. Visfatin by reduction of proliferation and the course of the cell cycle, may be an another important adipokine in the human placenta.

**Keywords:** visfatin, placenta, pregnancy hormones, proliferation, cell cycle

**Funding:** Diamond Grant IX 0110/DIA/2020/49. Monika Dawid was founded by finance from Doctoral Society at Jagiellonian University in Kraków.



**MARTA HITTA HERNANDEZ, PhD STUDENT**  
**UNIVERSITY OF PARIS-SACLAY GS LIFE SCIENCES AND HEALTH, FRANCE**

Marta is a PhD student in Reproductive Biology at the University of Paris-Saclay GS Life Sciences and Health. After getting her A levels in Madrid (Spain) she has started a degree in France at the University of Rennes 1 in Life Sciences where she was especially interested in molecular and cellular biology. She decided to do her last year of degree at the University of Bordeaux where she specialized in physiology and cellular and molecular biology. During her degree she realized several internships, the first at the National Center of Scientific Research of Rennes (CNRS) where she worked on oocyte maturation in mice, and the second at the University Hospital of Nantes where she worked on the cytogenetics service and more precisely on the preimplantation diagnosis service. In 2020 she received her MS in Cell Biology and Physio-pathology (specialty in Reproduction and Development) at the University of Paris Diderot and she begins her PhD about the molecular mechanisms of placental metabolic dysfunction during maternal obesity at the UMR 1198 BREED (RHuMA). During the first two years of her PhD she gained extensive experience in university teaching and supervised five students during their end-of-master internship. In addition, she has published her first scientific article as principal author on Reproductive Biology review and a second one in progress. Marta has also participated in national and international conferences (International Federation of Placental Association, Group de la Franconphonie Placentaire, Fédération Française d'Etude de la Reproduction...) with oral and poster presentations.



## Role of adiponectin on placental sphingolipid metabolism in maternal obesity

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**BACKGROUND:** Obesity is associated with dyslipidemia, chronic inflammation and oxidative stress, which could lead to a disruption in sphingolipid metabolism. Ceramides (a subgroup of sphingolipids) are signaling molecules involved in several processes such as cellular growth, differentiation, apoptosis, and in particular oxidative stress. Adiponectin, is known to control oxidative stress by regulating the expression of enzymes directly implicated in ceramide's catabolism. In this context, we studied the effect of maternal obesity (without gestational diabetes) on i) the placental oxidative stress and ii) the implication of adiponectin in the regulation of placental ceramide's metabolism.

**MATERIALS & METHODS:** Placental samples were collected from obese and lean women after cesarean delivery. Placental ceramide profile was analyzed by lipidomic approach using LC-MS/MS. Adiponectin receptor expression was assayed by transcriptomic and immunoblot analyses. Proteins modified by oxidative stress were studied by immunoblot experiment.

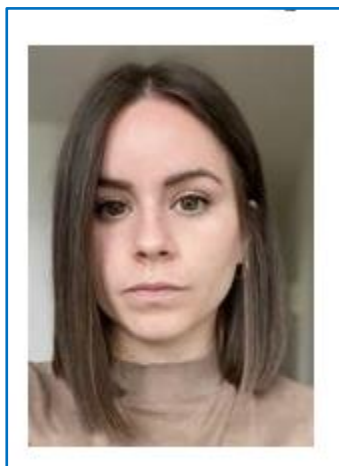
**RESULTS:** Our results showed i) a lower specific adiponectin receptor (AdipoR1/R2) expression, ii) a significant alteration of ceramide profile subgroups (lactosylceramide, dihydroceramide, and sphingomyelin), and iii) a lower expression of key enzymes of ceramide catabolism (FADS1/2) in the placenta from obese women. Preliminary results also demonstrated that maternal obesity seemed to increase placental oxidative stress as suggested by the apparition of a specific product only generated after lipid peroxidation.

**SUMMARY:** We hypothesized that the placental disruption of adiponectin signaling observed in obese women could lead to an alteration in placental ceramides' composition and so could explain the increase of placental oxidative stress. These alterations may alter the placental development and functions and consequently the health of the offspring.

**Keywords:** Placenta, obesity, adiponectin, ceramides, oxidative stress.

**Funding:** University funding (UVSQ)





**OPHELIE BERNARDI, PhD STUDENT  
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ENVIRONMENT, FRANCE**

Ophélie, PhD student (3rd year) on ChemPredict project in partnership with INRAE and SYSAAF in FRANCE. Its project aims to know if chemerin can be a potential biomarker of reproductive parameters in birds for poultry industry. Last 3 months, she participated at World's Poultry Congress at Paris, FRANCE. In collaboration with authors, she published the 1<sup>st</sup> article in *Frontiers in Physiology* (<https://doi.org/10.3389/fphys.2022.1012212>).

## Chemerin in egg albumen and cell number of germinal disc: potential biomarkers of the embryo development for genetic selection in birds

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**BACKGROUND:** One of the breeding companies goal is the production of viable and robust chicks. A major issue is the embryo mortality that represents 5 to 40% according to avian species. New fertility biomarkers are necessary to improve reproductive performances and genetic selection. We investigated two potential molecular markers of embryo development: chemerin concentration in albumen and the number of viable cells in the germinal disc at the oviposition time.

**MATERIALS & METHODS:** We studied two layer breeds, two broiler breeds and one duck breed. Eggs from 50 females were collected during one week laying at three periods of the laying cycle (before, after peak and at the end of laying period). For each egg, an albumen sample was collected to measure chemerin concentration by a home-made ELISA assay. The germinal disc was dissected to count viable cells. For each biomarker, the different breeds were compared by one-way ANOVA. Then, concentration of chemerin in albumen and viable cells number of germinal disc were correlated with reproductive performances by Pearson correlation.

**RESULTS:** The variability of chemerin concentration in albumen is higher inter-hen compared to intra-hen for each breed. We observed significant different concentrations of chemerin in albumen between breeds during the laying cycle. Chemerin concentration in albumen is positively correlated with laying, fecundity and fertility rates for layer breeds. Moreover, the number of viable cells of germinal disc is correlated: –negatively with laying and positively with fecundity rates for layers – positively with laying and hatchability rates for broiler –and negatively with fertility and hatchability rates for duck.

**SUMMARY:** Chemerin concentration in egg albumen and viable cells number of germinal disc may improve some reproductive parameters that's why we will study these biomarkers on a 2<sup>nd</sup> generation. These biomarkers could be involved in embryo development in chicken/duck and used in genetic selection.

**Keywords:** Adipokines, chemerin, egg albumen, germinal disk cells, biomarkers

**Funding:** This work was funded by INRAE, SYSSAF and CIFOG (ChemPredict, funding n°320001190)

## POSTER SESSION II ONLINE



**MARLENA GUDELSKA, PhD STUDENT**  
**UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN, POLAND**

Marlena received her M.Sc. in the Department of Animal Anatomy and Physiology at the University of Warmia and Mazury in Olsztyn, where she is currently a PhD Student. The team she works in is investigating the role of adipokines in the reproductive system of female domestic pigs. She completed an internship at the Jagiellonian University in Krakow. In her doctoral dissertation, she studies the role of chemerin in the uterus of domestic pig (*Sus scrofa domestica* L.) during the oestrous cycle and early pregnancy. Additionally, Marlena is the PI in the PRELUDIUM 16 project funded by the NCN, which aim is to understand the influence of resistin on the functioning of the pig uterus in the peri-implantation period. She participated in the implementation of 7 other projects carried out by the team. She participated in many scientific conferences organized, among others by Slovak University of Agriculture in Nitra, Society for Reproductive Biology or Polish Physiological Society. Marlena is a co-author in 23 papers published in renowned scientific journals. The publication: “*The expression of chemerin and its receptors (CMKLR1, GPR1, CCRL2) in the porcine uterus during the oestrous cycle and early pregnancy and in trophoblasts and conceptuses*”, in which she is the first author, was awarded by the Dean of the Faculty of Biology and Biotechnology UWM and Society for Reproductive Biology, of which she is a member. Her works have been cited 167 times and her h-index is 8.

## The role of chemerin in the uterus of domestic pig (*Sus scrofa domestica* L.) during the oestrous cycle and early pregnancy

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**BACKGROUND:** Chemerin, belonging to the adipokine family, has pleiotropic functions. Recent studies have revealed that chemerin may participate in the regulation of reproduction. The aim of this study was to determine the expression of chemerin system (chemerin and its receptors – CMKLR1, GPR1 and CCRL2), the impact of steroids (P<sub>4</sub> and E<sub>2</sub>) on the system expression and the effect of chemerin on the steroidogenesis process in the porcine uterus during the oestrous cycle and early pregnancy.

**MATERIALS & METHODS:** Basal chemerin system expression was determined in the pig uteri collected during the oestrous cycle (days: 2-3, 10-12, 14-16 and 17-19) and pregnancy (days: 10-11, 12-13, 15-16, and 27-28). Chemerin or steroids impact was determined on days 10-11 of the cycle and during early pregnancy. To define gene and protein expression real-time PCR and Western blot were used, respectively. Chemerin concentrations were measured using ELISA test, whereas steroid hormones levels were defined by radioimmunoassay.

**RESULTS:** We have found that the expression of the chemerin system in the porcine uterus is dependent on the oestrous cycle phase and/or early pregnancy period. We also found that P<sub>4</sub> and E<sub>2</sub> modulate the chemerin system expression. Moreover, we found that the adipokine influences the steroidogenesis process in the uterus.

**SUMMARY:** The obtained results suggest that chemerin may be a link between metabolic status and reproduction of female pigs. The adipokine may be a crucial factor in the regulation of the secretory functions of the porcine uterus, an economically important species.

**Keywords:** chemerin, uterus, steroids, steroidogenesis, endometrium, myometrium

**Funding:** This research was supported by the Polish National Science Centre (project no 2017/25/B/NZ9/00040).



**GRZEGORZ KOPIJ, PhD student**

**UNIVERSITY OF WARMIA AND MAZURY, POLAND**

Grzegorz is a PhD student in the field of Biological Sciences in the Discipline of Natural Sciences in the Department of Animal Physiology and Anatomy at the University of Warmia and Mazury in Olsztyn. He has a member of the Society of Reproductive Biology. He completed training in monitoring clinical trials. The team he works with and realizes his doctoral thesis investigates the effect of adipocytokines on the reproductive system, including elements of the HPG axis in female domestic pigs. During his education at the Doctoral School, he was a scholarship holder in two research projects (2017/25/BNZ9/00040; 2018/31/BNZ99/00781) and from December 2021 he is the PI in the Preludium-20 project financed by the NCN (2021/41/N/NZ9/02325). His doctoral dissertation aim to evaluate the impact of visfatin on the transcriptomic and proteomic profiles of the luteal cells of the pig (*Sus scrofa domestica* L.) during the oestrus cycle and early pregnancy. As part of the project Preludium-20, he studies the expression of the obestatin gene and protein in the porcine uterus. He also examines the impact of obestatin on the steroidogenesis in the porcine endometrium and the adipokine impact on the transcriptomic and proteomic profiles of the luminal epithelial cells of the porcine uterus. Grzegorz is a co-author in 11 papers published in scientific journals from JCR list and his h-index is 10.

## Visfatin impact on the transcriptome of luteal cells of domestic pig (*Sus scrofa domestica* L.) during early pregnancy

KOPIJ G<sup>1</sup>, KIEZUN M<sup>1</sup>, DOBRZYN K<sup>2</sup>, ZAOBIDNA E<sup>3</sup>, KAMINSKA B<sup>1</sup>, RAK A<sup>4</sup>, KAMINSKI T<sup>1</sup>, SMOLINSKA N<sup>1</sup>

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**BACKGROUND:** Our preliminary data indicate that visfatin has a significant effect on the reproductive system of female pigs. We assumed that visfatin may affect the global transcriptome of luteal cells and thus influence the ovarian functions.

**MATERIALS & METHODS:** RNA-sequencing was performed to investigate the differentially expressed genes (DEGs), differential alternative splicing events (DASEs) and long non-coding RNAs (lncRNAs) in the cultured porcine luteal cells collected during the implantation period (days 15-16 of gestation) and exposed to visfatin (VIS; 100 ng/mL) for 24 hours. High-throughput sequencing of transcriptomes was performed on the Illumina NovaSeq 6000 platform.

**RESULTS:** NGS analysis revealed 102 DEGs: 58 were upregulated and 44 were downregulated in the VIS exposed group. We identified 252 DASEs, and 40 lncRNAs, of which 3 are known lncRNAs and 37 novel lncRNAs. 23 lncRNAs were upregulated and 17 were downregulated.

**SUMMARY:** Visfatin has an impact on transcriptome of pig luteal cells, including genes which products are involved in angiogenesis, steroidogenesis, inflammation, cell development, migration and proliferation. In conclusion, visfatin, *via* its influence on ovarian transcriptome may play a key role in the modification of physiological processes connected to reproduction.

**Keywords:** adipokine, visfatin, transcriptome, pig, pregnancy, ovary, luteal cells

**Funding:** This research was supported by the Polish National Science Center OPUS 16 2018/31/B/NZ9/00781





SERRA LOÏSE, PhD STUDENT  
NATIONAL RESEARCH INSTITUTE FOR AGRICULTURE,  
FOOD AND THE ENVIRONMENT, FRANCE



Loïse, PhD student in the SENSOR team at INRAE, in Nouzilly (France) working on the effect of pesticides on fertility. She worked during her master degree on the uterine transplantation in the BREED team at INRAE, in Jouy-en-Josas (France) and more specifically on the sheep model and the biomarkers of ischemia/reperfusion after an auto-transplantation. During her PhD with Dr Joëlle Dupont and Dr Pascal Froment, she is interested by endocrine disruptors such as herbicides, insecticides and fungicides and their impact on the fertility of human, mice and chicken species. She has already published a review about the fertility and glyphosate-based herbicides (<https://doi.org/10.3390/cells10113079>) as well as an article about the fertility of rooster and chronic dietary exposure to glyphosate-based herbicide by food (<https://doi.org/10.3390/toxics9120318>).

## Effects of endocrine disruptors on adipokine secretions

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**BACKGROUND:** Glyphosate is largely used as the active ingredient of commercial herbicides such as Roundup (RU). Its potential as endocrine disruptor has been described in the literature. However, no studies have been performed about its impact on adipokine secretion related to the reproduction system such as Chemerin. In a previous study of our laboratory, Chemerin was showed to be negatively correlated to the sperm motility in roosters.

**MATERIALS & METHODS:** Adult roosters were exposed from the diet to RU (46.8 mg equivalent glyphosate/kg body weight/day) for 36 days, and then received control food for 14 days. Seminal fluid and blood plasma were collected. Glyphosate, AMPA (amino-methyl-phosphonic acid), its metabolite, and Chemerin were assayed in the seminal fluid and in the plasma during the exposure and after, compared to the control group. Sperm parameters were analyzed by computer-assisted sperm assessment (CASA) using IVOS (Integrated visual optical system).

**RESULTS:** Glyphosate and AMPA were increased in the seminal fluid compared to the blood plasma during the exposure. The motility of spermatozoa was decreased during the exposure compared to the control group. Seminal fluid chemerin was increased during the exposure in the RU group. These negative effects were abolished 14 days after RU removal from the diet.

**SUMMARY:** Chronic dietary RU exposure in roosters caused accumulation of glyphosate and AMPA in the seminal fluid. It also enhanced the secretion of seminal fluid chemerin and reduced the sperm motility. All these effects were reversible after the end of the exposure.

**Keywords:** Glyphosate, Roundup, fertility, chemerin, roosters, endocrine disruptors.

**Funding:** The authors thank to Région Centre Val de Loire for this funding (HAPOFERTI grant number 32000858).