



**Institute of Animal Reproduction and Food Research
of the Polish Academy of Sciences**

Publication distributed free of charge

Graphic Design & DTP:
www.partnersstudio.com

Publication co-financed by the REFRESH Project



"Unlocking potential of the Institute of Animal Reproduction and Food Research
for reinforced ERA integration and regional development"



Research Potential - Capacities - 7th Framework Programme
EU FP7-REGPOT-2010-1-264103



Institute of Animal Reproduction and Food Research
of the Polish Academy of Sciences

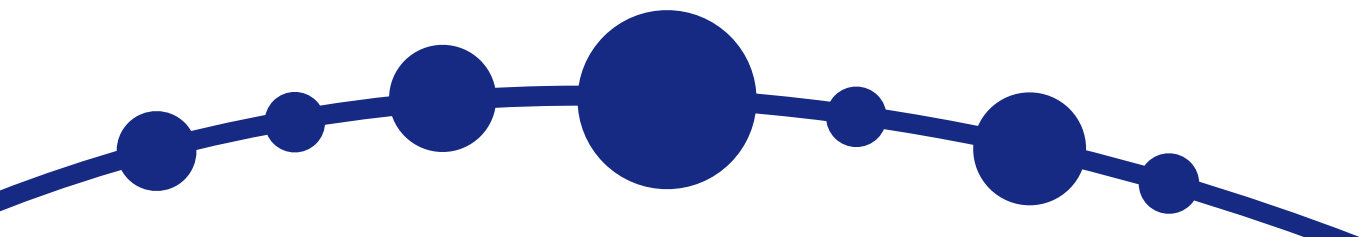




Table of contents

Table of contents

Preface	6
Institute's history	8
About us - Institute of Animal Reproduction and Food Research	11
Division of Reproductive Biology	14
Department of Hormonal Action Mechanisms	14
Department of Reproductive Immunology and Pathology	18
Department of Physiology and Toxicology of Reproduction	22
Department of Local Physiological Regulations	26
Department of Gamete and Embryo Biology	30
Department of Biology and Pathology of Human Reproduction	34
Division of Food Sciences	38
Department of Immunology and Food Microbiology	38
Department of Chemistry and Biodynamics of Food	42
Department of Chemical and Physical Properties of Food	46
Department of Biological Function of Food	50
Department of Biosensors	54
Integrated Laboratory of Bioelectroanalysis	58
Department of Prophylaxis of Metabolic Diseases	62
Laboratories	66
In Vitro and Cell Biotechnology Laboratory	66
Molecular Biology Laboratory	68
Microbiological Laboratory	70
Sensory Laboratory	72
Animal Laboratory	74
The Welcome Project Team	76
Institute in recent international projects	80





Preface

Preface

This Guide Book represents a further update about the Institute's activities. As always on such an occasion it is necessary to refer to the past. From its very beginning the Institute has developed at an impressive pace. Those who created it and guided it initially are now handing over the reins to the next generation. Without their vision and their belief that this undertaking could, with a great deal of time and effort, become a reality, the establishment of a new scientific centre, our Institute, would not have been possible.

From the original Centre for Agrotechnology and Veterinary PAS in Olsztyn, which consisted of a small group of dedicated enthusiasts and a stamp, we have evolved to the present Institute which employs over 170 people, across three sites which, in total, approach 6 thousand square metres. It is impossible here not to mention the names of its founders and first people leading the Institute - Professor Halina Kozłowska, Professor Tadeusz Krzymowski, Professor Antoni Rutkowski and our, until recently, long-term Director Professor Adam Zięcik. To these illustrious names must be added the involvement of generations of young, hard-working and ambitious scientists and an insatiable desire to establish and exploit international cooperation. Together this is the reason for today's high position and reputation of the Institute.

Not so long ago, in celebration of the Institute's 15th anniversary, we proudly announced that, as a national leader in agricultural sciences, the scientific papers published by the Institute were cited 200 times a year. Today, after less than 10 years, such achievements distinguish several of the Institute's leading researchers. It is these research leaders who, just like the Institute's founders, guide, train and inspire others, represent the strength of today's Institute.

The excellence of the research being carried out and the ability to be ahead of scientific trends, these represent another reason for our success. Of course, this has and will continue to have a considerable influence on the priority directions of research conducted in the Institute, which now focus on:

- recognising of the proper functioning of the processes taking place in the reproductive tract;
- elucidating the causes of infertility and reproductive disturbances in reproduction of humans and animals, including early embryonic mortality;
- introducing therapeutic techniques, reproductive bio-techniques and biotechnological methods to prevent and cure infertility, protect the biodiversity of animal production and se-



- lected endangered species;
- improving food and nutrition to preserve human health and well-being;
- identifying food and environmental influences on the prophylaxis of allergies, obesity, type 2 diabetes, and other diet-related disorders;
- implementing scientific research and development activities to increase the pro-health value of food.

It can be seen that the directions of research conducted in the Institute have developed and, in comparison to their original scope, have broadened within the area known as human health and well-being.

The excellence of the scientific research carried out in the Institute's two fundamental fields of activity - animal reproduction and food research - has been recognized at both the national and European level and has been confirmed through the status of "Centre of Excellence" which has been awarded to both areas.

In addition to the dedication and enthusiasm of its staff, the Institute has extensive state-of-the-art analytical facilities enabling its research to be carried out at the highest international level. This combination of scientists and facilities is a particularly strong argument when applying for funds for research projects, engaging in partnerships with scientific units or developing its recently-established collaboration with industry. The last few years have been exceptionally successful in this respect. Funding has been secured from a wide range of sources to support and extend our research infrastructure, so that now we can say, with no exaggeration, that it equals similar European scientific centres.

I am sure you will see this yourself after reading this new and updated guide book of our Institute.

Director General
Prof. Mariusz K. Piskula



Institute's history



Institute's history

1988 - foundation of the Centre for Agrotechnology and Veterinary Sciences of the Polish Academy of Sciences with two Departments:

- Department of Food Technology,
- Department of Reproductive Endocrinology;

1992 - change of the organizational structure - establishing two Divisions:

- Division of Food Science,
- Division of Reproductive Endocrinology and Pathophysiology;

1994 - obtaining the right to confer a doctoral degree of Agricultural Sciences in the major of:

- Food Technology and Nutrition,
- Animal Husbandry;

1995 - change of the Centre status into the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences;

1998 - opening a new laboratory building of the Division of Food Science located at 10 Tuwima Str.;

1999 - obtaining the right to confer the habilitation degree in the major of:

- Food Technology and Nutrition,
- Animal Husbandry;

2000 - termination of the research activity of the Department of Developmental and Experimental Endocrinology in Poznań and the Department of Sensory Analysis of Food in Warsaw;

2001 - obtaining the status of European Union Centre of Excellence in the area of FOOD & HEALTH;

2002 - obtaining the status of National Centre of Excellence in the area of ANIMAL REPRODUCTION;

2007 - opening a new laboratory building of the Division of Reproductive Endocrinology and Pathophysiology located at 7 Bydgoska Str.;

2008 - change of the organizational structure (reorganization of Departments through their merging or establishing new Laboratories);

- Division of Food Sciences,
- Division of Reproductive Biology;





2010 - establishment of two medical profile Departments of the Institute in Białystok:

- Department of Prophylaxis of Metabolic Diseases,
- Department of Biology and Pathology of Human Reproduction;

2010 - implementation of the EU FP 7th REFRESH Project, entitled "Unlocking potential of the Institute of Animal Reproduction and Food Research for reinforced ERA integration and regional development";

2013 - 25th anniversary of the Institute.



The function of Institute Director General was performed by:

Prof. Tadeusz KRZYMOWSKI - PAS full member (1988 - 1997)
 Prof. Halina KOZŁOWSKA (1997 - 2000)
 Prof. Adam J. ZIĘCIK - PAS corresp. member (2000 - 2004; 2004 - 2008; 2008 - 2012)

The function of Institute Deputy Director for Science was performed by:

Prof. Halina KOZŁOWSKA (1988 - 1997)
 Prof. Adam ZIĘCIK - PAS corresp. member (1992 - 1995, 1998 - 2000)
 Prof. Jan KOTWICA - PAS corresp. member (1995 - 1998)
 Prof. Henryk KOSTYRA (1997 - 2004)
 Prof. Jan GLOGOWSKI (2000 - 2004)
 Prof. Józef FORNAL (2004 - 2007)
 Prof. Dariusz SKARŻYŃSKI (2004 - 2008, 2008 - 2012)
 Prof. Mariusz PISKUŁA (2007 - 2012)

The function of Chairman of the Institute Scientific Board was performed by:

Prof. Antoni RUTKOWSKI - PAS full member (1988 - 1998)
 Prof. Tadeusz KRZYMOWSKI - PAS full member (1999 - 2002)
 Prof. Zbigniew ŚMIETANA (2003 - 2006)
 Prof. Jan KOTWICA - PAS corresp. member (2007 - 2010)

The function of vice Chairman of the Institute Scientific Board was performed by:

Prof. Adolf HORUBAŁA - PAS full member (1988 - 1998)
 Prof. Tadeusz GARBULIŃSKI - PAS full member (1988 - 1996)
 Prof. Wiesław BAREJ - PAS corresp. member (1996 - 1998)
 Prof. Antoni RUTKOWSKI - PAS full member (1999 - 2002)
 Prof. Jan KOTWICA - PAS corresp. member (1999 - 2002; 2003 - 2006)
 Prof. Ryszard AMAROWICZ (2003 - 2006; 2007 - 2010)
 Prof. Jadwiga PRZAŁA (2007 - 2010)

At present, the Institute is directed by:

Prof. Mariusz K. PISKUŁA - Director General
 Prof. Barbara WRÓBLEWSKA - Deputy Director for Science
 Prof. Dariusz SKARŻYŃSKI - Deputy Director for Science

The function of the Chairman of the Institute Scientific Board is performed by:

Prof. Włodzimierz BEDNARSKI - PAS corresp. member

The function of vice Chairman is performed by:

Prof. Ryszard AMAROWICZ - Chair of the Food Sciences Commission
 Prof. Andrzej CIERESZKO - Chair of the Biology of Reproduction Commission



About us - Institute of Animal Reproduction and Food Research



About us - Institute of Animal Reproduction and Food Research

Mission

IAR&FR's mission is to conduct fundamental and applied research in the area of food, biology of reproduction and health. The activities that complement this objective include training scientists, making research findings available to society and bridging the gap between science and industry to foster the socio-economic development of Eastern Poland. Institute's multidisciplinary research staff of around 140 along with 30 PhD students, operate in 12 research departments to solve issues of fundamental importance to the regional and global challenges of:

Food Quality and Safety through its research on the mutual interaction between food components, the food matrix and the human body; identification, assessment and implementation of strategies for increased nutritional and health value of food; as well as in-depth understanding of the nature, implications and treatment of adverse reactions of consumers to dietary components - including intolerance, allergenicity and pathogenicity;

Biology of Reproduction through its scientific investigations of physiological and immunological mechanisms regulating the ovarian cycle and pregnancy in humans and animals; reproductive biochemistry and biotechnology; as well as developing tools for the protection of the biodiversity of animal production and selected endangered species;

Health in respect of the attainment and maintenance of human well-being with the emphasis put on the identification of food and environmental influences on the prophylaxis of obesity, type 2 diabetes, allergies and other diet-related disorders; investigations of the causes of infertility and disturbances in reproduction of humans and animals and introduction of new therapeutic techniques and biotechnological methods to prevent and cure infertility.

Training and career development

IAR&FR is a powerful supplier, mainly through its PhD and a two-way secondment programme, of highly-skilled staff for the food, biology of reproduction and health commercial and academic centres. Our graduate studies aim at enabling the effective completion of young scientists' research tasks as well as at providing them with cross-disciplinary knowledge crucial for tackling the rising challenges within the field of life sciences. Part-

nerships established with world-recognized research centres across the entire Europe allow for the twinning visits of scientists and a successful exchange of know-how. Such two-way secondment initiatives improve the professional qualifications of scientists and foster the development of common areas of interest that result in international joint research projects.

IAR&FR's science

Research in IAR&FR concerns:

- evaluation of food components and their interactions with the organism of a consumer;
- the effects of nutrients and non-nutrients on the sensory and functional properties of food, its safety, quality and pro-health value;
- influence of genetic, epigenetic and environmental factors, including diet, ambient temperature, environmental pollution, on the regulation of physiological processes;
- allergenic and immunogenic functions of natural and modified food components;
- pathological disturbances in the female reproductive tract, inflammation and degenerative processes in humans and animals;
- reproductive biotechnology and biochemistry of domestic and wild animals - including local breeds of domestic animals and individual species of fish and birds;
- hormonal, immune-endocrinal, neural and vascular regulation of the ovarian cycle and the mechanisms regulating communication between gametes and a mother.

Cooperation with industry

One of the IAR&FR's main focuses is to communicate its science for the benefit of society and industry. With this aim at heart we are engaging in a dialogue with both - knowledge users, i.e. manufacturers, entrepreneurs and consumers, and knowledge generation funders such as organisations and governmental bodies providing subsidies for the research. By leading theme-oriented seminars, workshops and brokerage meetings we foster interchange with agri-food industry and provide a direct link for the IAR&FR science programmes to the commercial sector. Many challenges the industry is currently facing can only be addressed by merging skills and expertise from various scientific areas. Bringing together the disciplines of Food Research and Biology of

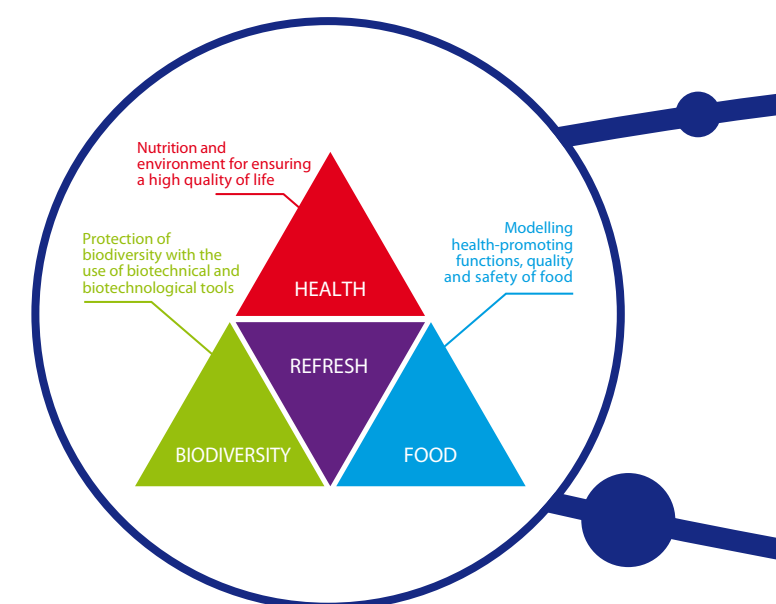
Reproduction, the Institute is uniquely positioned to provide our industrial partners with a wide array of multidisciplinary tailor-made solutions.

Among other, our offer includes a complex analysis of raw materials and products, evaluation of the influence of storage and technological processes on the quality, safety and attractiveness of food. We conduct consumer and analytical sensory research, benchmarking of food materials and products, consumer preference mapping that proves to be a valuable source of information about sensory properties of foods, as well as about psychological and behavioural motivations of consumers. What is more, acknowledging that quality and safety of food is strongly dependent on the quality of raw materials composing it, we enhance the reproductive quality and effectiveness of livestock and fish. We use innovative research tools enabling us to understand the mechanisms influencing the regulation and control of the reproduction process. We search for new and alternative methods for treating disturbances in reproduction, rearing and breeding. To go one stage further, we offer the possibility of cryopreservation of animal and fish sperm, contributing to the ex situ protection of endangered species and maintenance of sustainable biodiversity.

Engaging in sustainable socio-economic development

Home of IAR&FR, Warmia and Mazury Voivodeship, is one of the least economically developed regions of Poland. It is marked by high unemployment, poor infrastructure conditions and early-stage regional system for the support of technological development and transfer to SME sector. Recognizing these challenges, but also strong in-

novation potential of many agri-food industries, the Institute of Animal Reproduction of Food Research has leveraged national and regional funds to develop projects aimed at raising the social and economic sustainability of Warmia and Mazury. The "Regional Centre for Research and Transfer of Knowledge Food for Health" increases the competitiveness of the regional economy through creation of advanced research facilities for the execution of innovative research and effective transfer of results into practice. "Knowledge and Region Development" seminars contribute to popularization of science and facilitate the dialogue between science, industry and general public for the development of innovation and technology. The "Liaison Officer" Project reflects IAR&FR's strong commitment to economic impact by establishing partnerships with entrepreneurs, helping them to solve problems and exploit commercial opportunities.



REFRESHing research potential

The European Union Commission has recognized the leading position of IAR&FR in the area of agricultural sciences, by entrusting the Institute with a realization of a 3.6 million Euro project called "REFRESH - Unlocking the potential of the IAR&FR for reinforced ERA integration and regional development".

The objective of REFRESH is to implement a new strategy of Institute's development through investment in research areas focusing on BIODIVERSITY, FOOD and human HEALTH. Above all, REFRESH will enhance the research potential of PAS in Olsztyn by upgrading its analytical facilities, recruitment of top-class scientists, targeted secondments of IAR&FR's researchers, twinning with leading research centres and supporting the organisation of international conferences.

Once fully established, REFRESH will facilitate the translation of knowledge to local authorities, agri-food industries and general public, and initiate active involvement in actions that will stimulate the socio-economic development of the Eastern Poland.

Division of Reproductive Biology

Department of Hormonal Action Mechanisms

Division of Reproductive Biology
Bydgoska 7 Str., 10-243 Olsztyn



Head
Agnieszka Blitek, Ph.D., D.Sc.

Professors
Adam J. Zięcik, Prof.
Aneta Andronowska, Ph.D., D.Sc.

Assistant Professor
Agnieszka Wacławik, Ph.D.

Assistant
Marcin Chruściel, Ph.D.

Senior Specialists
Katarzyna Gromadzka-Hliwa, M.Sc.
Jan Kłos, M.Sc.
Benigna Szerejko, M.Sc.
Zbigniew Lipiński, Ph.D.

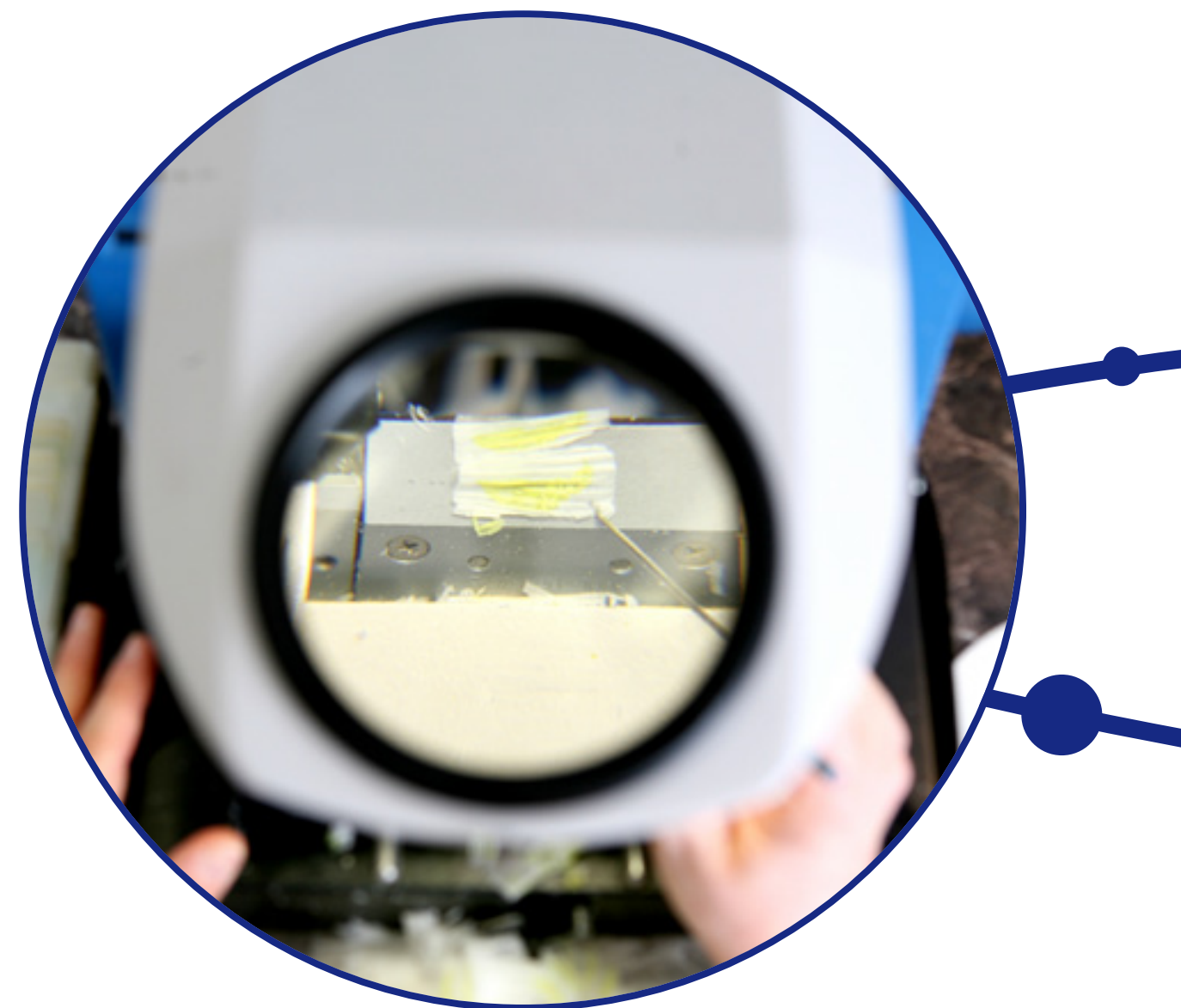
Senior Technician
Jadwiga Sułek

Assistant in the project
Marcelina Łopińska, M.Sc.

Ph.D. Students
Kamil Krawczyński, M.Sc.
Ewa Morawska-Pucińska, M.Sc.
Piotr Kaczyński, M.Sc.
Izabela Małysz-Cymborska, M.Sc.
Emilia Bołzan, M.Sc.

Focus areas

Embryo-uterine interactions during the maternal recognition of pregnancy and implantation as well as factors involved in the control of early embryonic development are the main scientific interests of the Department of Hormonal Action Mechanisms. The majority of the embryonic mortality in swine occurs before 30th day of gestation. It is not surprising when taking into consideration the crucial events that take place during this period, e.g. embryo migration, rapid differentiation and expansion of conceptuses, the process of maternal recognition of pregnancy, trophoblast attachment to the uterine wall and placentation. Reasons for the early pregnancy failure may be of conceptus (genetic factors, weak conceptus signalling) or maternal (impaired uterine receptivity) origin. Thus, orchestrated synchronization between embryo development and endometrial receptivity are essential for successful pregnancy establishment. It became obvious that the action of progesterone in priming the uterus is absolutely necessary to attain uterine receptivity. Therefore, sustained function of corpora lutea is of high importance for the establishment and maintenance of pregnancy. Luteal deficiency has been indicated as one of the reasons of fetal losses detected at 40-50th day of pregnancy in pigs. What is more, exogenous hormones administered to advance puberty and/or to control estrous cycles may negatively affect the function of female reproductive tract and the quality of oocytes/embryos. Understanding of embryo-maternal cross-talk and determination of factors associated with conceptus development might be helpful



in the development of new methods of animal selection for reproduction and may improve embryonic survival. Search for and the use of markers of reproductive efficiency would be of great importance for the increase in the pig production, but also for transgenesis, cloning and other biotechniques.

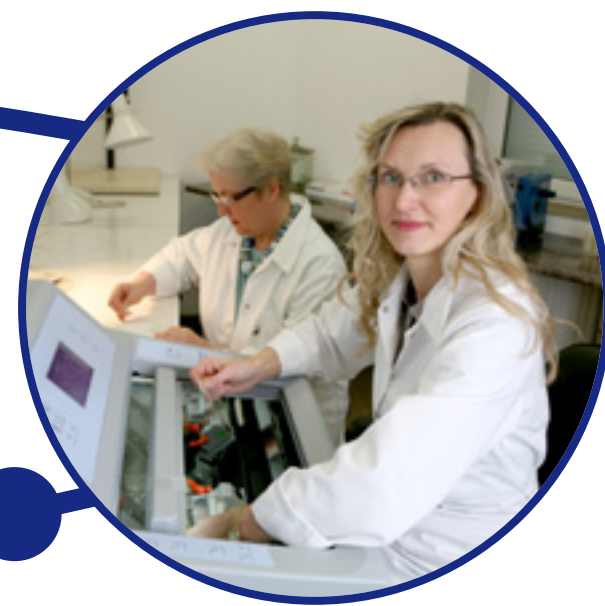
Current research

1. Analysis of the mechanism of para- and autocrine action of prostaglandin E2 (PGE2) and PGF2 α on embryos and the uterus during early pregnancy, as well as determination of the role of prostaglandin synthesis enzymes and prostaglandin receptors during maternal recognition of pregnancy and implantation;
2. Determination of cytokines and morphogenes involvement in embryo-uterine interactions

during periimplantation period. The projects aims to determine the effect cytokines exert on conceptus trophoblast and uterine epithelial cells, and to analyze functional connections between WNT and HOXA genes in the porcine endometrium;

3. Studies on luteolytic sensitivity in pigs undertaken to determine molecular mechanisms involved in this process. The aim of the project is to identify genes associated with overcoming luteal regression during early pregnancy and to determine the period and the way corpus luteum can be rescued from regression. Results will be useful in elaboration of new pharmacological methods enhancing luteal function;
4. Examination of the role of prostacyclin and its receptors in the establishment of preg-

- nancy in the pig. The project involves determination of factors that regulate prostacyclin synthesis, as well as a study of the effect of prostacyclin on trophoblast, endometrial and luteal cell functions;
5. Evaluation of the effect of superovulation on VEGF-receptors system in the oviduct after fertilization;
 6. Development of a new method for reducing embryo mortality in pigs by limitation of luteal dysfunction. The objective of the studies is to determine the composition of hormonal preparation that will imitate embryonic signals, as well as optimal period of its administration during early gestation. These hormonal preparations may be useful in pig production and after embryo transfers.



Achievements

- cloning and sequencing of the porcine PGF synthase and PGE2 synthase cDNAs and characterization of their expression in the endometrium and conceptus/trophoblast;
- demonstration of the presence of PGE2 positive feedback loop in the porcine endometrium at the time of the maternal recognition of pregnancy. Primary conceptus signal, E2, and conceptus-derived PGE2 regulate the expression of prostaglandin synthesis pathway enzymes to favor the production of luteoprotective PGE2 in the endometrium;
- demonstration of HOXA10 gene expression in the uterus of adult pigs during periimplantation period; ovarian steroids and conceptus

products are important regulators of HOXA10 expression in endometrial cells;

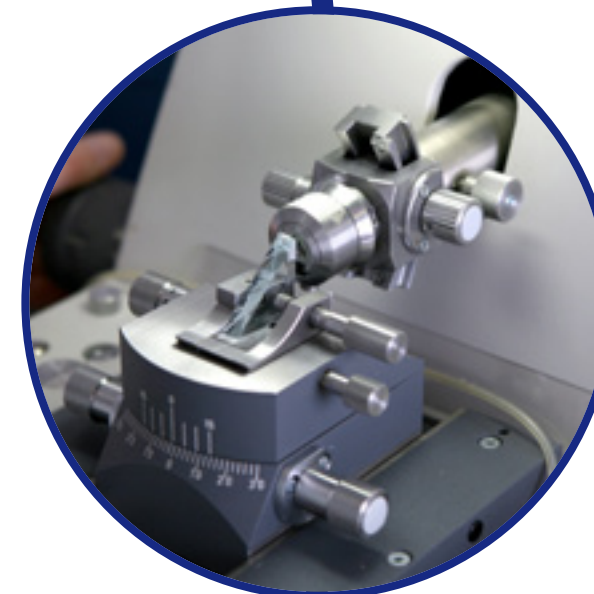
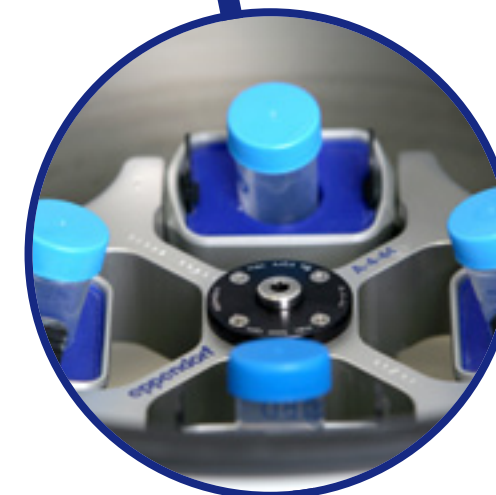
- determination of vascular endothelial growth factor (VEGF) and its receptors expression in the porcine uterus during the estrous cycle and early pregnancy, which suggests an important role of VEGF-receptor system implantation process and placenta development.

Selected papers

1. Kiewisz J., Kaczmarek M.M., Morawska E., Blitek A., Kapelanski W., Ziecik A.J. (2011) Estrus synchronization affects WNT signaling in the porcine reproductive tract and embryos. *Theriogenology* 76: 1684-1694.
2. Waclawik A. (2011) Novel insights into the mechanisms of pregnancy establishment: regulation of prostaglandin synthesis and signaling in the pig. *Reproduction* 142: 389-399.
3. Chrusciel M., Bodek G., Kirtiklis L., Lewczuk B., Hyder C.L., Blitek A., Kaczmarek M.M., Ziecik A.J., Andronowska A. (2011) immortalization of swine umbilical vein endothelial cells (SUVECs) with the simian virus 40 large-T antigen. *Molecular Reproduction and Development* 78: 597-610.
4. Blitek A., Morawska E., Kiewisz J., Ziecik A.J. (2011) Effect of conceptus secretions on HOXA10 and PTGS2 gene expression, and PGE2 release in co-cultured luminal epithelial and stromal cells of the porcine endometrium at the time of early implantation. *Theriogenology* 76: 954-966.
5. Kiewisz J., Kaczmarek M.M., Andronowska A., Blitek A., Ziecik A.J. (2011) Gene expression of WNTs, β -catenin and E-cadherin during the periimplantation period of pregnancy

in pigs – involvement of steroid hormones. *Theriogenology* 76: 687-699.

6. Blitek A., Waclawik A., Kaczmarek M.M., Kiewisz J., Ziecik A.J. (2010) Effect of estrus induction on prostaglandin content and prostaglandin synthase expression in the uterus of early pregnant pigs. *Theriogenology* 73: 1244-1256.
7. Waclawik A., Blitek A., Ziecik A.J. (2010) Oxytocin and tumor necrosis factor alpha stimulate expression of prostaglandin E2 synthase and secretion of prostaglandin E2 by luminal epithelial cells of the porcine endometrium during early pregnancy. *Reproduction* 140: 613-622.
8. Kaczmarek M.M., Krawczynski K., Blitek A., Kiewisz J., Schams D., Ziecik A.J. (2010) Seminal plasma affects prostaglandin synthesis in the porcine oviduct. *Theriogenology* 74: 1207-1220.
9. Blitek A., Kaczmarek M.M., Kiewisz J., Ziecik A.J. (2010) Endometrial and conceptus expression of HoxA10, transforming growth factor beta1, leukemia inhibitory factor, and prostaglandin H synthase-2 in early pregnant pigs with gonadotropin-induced estrus. *Domestic Animal Endocrinology* 38: 222-234.
10. Waclawik A., Jabbour H.N., Blitek A., Ziecik A.J. (2009) Estradiol-17beta, prostaglandin E2 (PGE2), and the PGE2 receptor are involved in PGE2 positive feedback loop in the porcine endometrium. *Endocrinology* 150: 3823-3832.



reproduction, hormonal control, early pregnancy, prostaglandins, cytokines, receptivity markers, pig

Contact
Agnieszka Blitek, Ph.D. Sc.
a.blitek@pan.olsztyn.pl

Collaboration partners

- Department of Physiology, University of Turku, Finland;
- Medical Research Council Human Reproductive Science Unit, the Queens Medical Research Institute, Edinburgh, United Kingdom.

Department of Reproductive Immunology and Pathology

Division of Reproductive Biology
Bydgoska 7 Str., 10-243 Olsztyn



Head

Prof. Dariusz J. Skarżyński, DVM, Ph.D., D.Sc.

Associated Professor

Barbara Jana, DVM, Ph.D., D.Sc.
Izabela Woźniak-Potocka, DVM, Ph.D., D.Sc.

Assistant Professors

Anna Korzekwa, Ph.D., D.Sc.
Marta Siemieniuch, DVM, Ph.D.

Post-doc Fellow

Antonio M. Galvao, DVM, Ph.D.

Senior Specialists

Mamadou M. Bah, DVM, Ph.D.
Katarzyna Jankowska, Ph.D.

Technologists

Karolina Łukasik, M.Sc.
Ilona Kowalczyk-Zięba, M.Sc.
Witold Krzywiec
Joanna Kalinowska

Ph.D. Students

Anna Szóstek, M.Sc.
Edyta Brzezicka, M.Sc.
Katarzyna K. Piotrowska-Tomala, DVM
Dorota Boruszewska, M.Sc.
Joanna Czarzasta, M.Sc.
Ewelina Jursza, M.Sc.

Focus areas

The main research activities carried out in the Department of Reproductive Immunology and Pathology focus on: (1) immune-endocrine and molecular mechanisms involved in estrous cycle, pregnancy and birth regulation in farm animals (mares, cows and pigs), domestic and wild animals (felines); (2) immune-endocrine mechanisms regulating communication between embryo and mother in the early stage of pregnancy on cellular, receptor and molecular level; (3) immune-endocrine and neurological changes accompanying pathological disturbances in females reproductive organs and mammary gland; (4) participation of peripheral nervous system and immune system in pathophysiology of cystic ovaries degeneration; (5) receptor, intracellular and molecular mechanism of actions of hormones, cytokines and other biologically active substances and their analogues for potential using in the control of the estrous cycle and early pregnancy, as well as potential therapeutics in disorders of peri-parturient period in humans and animals.

Realization of the main scientific objectives of the Department is accompanied by the performance of applied studies that focus on: (1) elucidating the causes of the high incidence of early embryonic mortality in domestic animals; (2) introduction new therapeutic, biotechnological methods and biotechniques for the improvement of reproductive efficiency in domestic animals; (3) creation of new alternative, for chemotherapy, strategies/drugs in the diagnostics and therapy of the reproductive system and mammary gland pathologies (neoplasia, inflammations, etc.), what in turn may allow to restrict the creation of bacteria resistance to chemotherapeutics;



(4) creation of new methods and strategies for the limitation of reproduction in animals the increasing population of which can bring sanitarian and epidemiological risk or disturb the natural ecosystem (un-invasive pharmacology castration of carnivores, etc.); (5) development of new methods of reproductive biotechnology and biotechniques to protect endangered wild species including feline and local breeds of domestic animals.

Current research

1. Molecular, cellular and immune-endocrine mechanisms of endometrial fibrosis: mare uterus as a research model;
2. Effect of prostaglandin F2alpha on secretion and action of fibroblastic growth factor in bovine corpus luteum: participation in the regulation of angiogenesis and luteolysis;
3. Immuno-endocrine disorders of corpus luteum regulation as a potential cause of early pregnancy loss in mares and companion animals;
4. Secretion and the role of leukotrienes in porcine uterus during experimentally induced inflammatory processes: *in vitro* and *in vivo* studies;
5. Is placenta involved in the hormonal homeostasis during pregnancy in cats?;
6. Role of tumor necrosis factor-alpha in pathogenesis of lipopolysaccharide-induced endometritis-pyometra syndrome in domestic cat - *in vitro* studies;
7. Adenomyosis in cattle: molecular mechanism of generation and factors modifying development of the disease;
8. Neutrophil extracellular traps and cytokines in the equine endometrium: friends or foes?

Achievements

- showing that nitric oxide (NO) is the main auto/paracrine mediator of luteolytic actions of PGF2α and cytokines during corpus luteum (CL) regression in cow and mare. NO inhibits progesterone (P4) secretion, stimulates prostaglandin production and induces CL cells apoptosis during luteolysis;
- demonstration and description of pro-inflammatory cytokines (tumour necrosis factor-α, interferon-γ, Fas-L), as local (auto/paracrine factors) modulate secretory functions of the bovine, equine and feline CL and uterus. Interleukine-1α acts mainly as a luteotropic factor supporting the action of the embryo signal - interferon-τ during the early pregnancy in cow;
- determining that lysophosphatidic acid and leukotrienes play the auto-paracrine role in

the bovine ovary and uterus during the estrous cycle and early pregnancy;

- description of cellular, enzymatic and molecular mechanisms of pro-inflammatory cytokines, NO and bacterial endotoxin (LPS) actions in the pathogenesis of inflammation of the porcine and bovine uterus and bovine mammary gland. Production and output of PGE2, PGF2 α and leukotrienes are modulated in inflamed tissues;
- revealing that in the cystic ovaries in pigs a significant increase in noradrenergic nerves and catecholamine concentration goes with the changes in steroidogenic activity. The increase of noradrenergic nerves is dependent on the expression of nerve growth factor.



Analytical facilities

1. Two workstations for isolation and cells culture: Laminar chambers (Thermo Scientific MSC-ADVANTAGE'180 and 120, Polon'60), CO2Incubators (ThermoForma, Sanyo), Centrifuges (MPW 350R, MPW 223E), Peristaltic pumps, Inverted microscopes (Olympus CKX41), Incubators with shaker (EB TH15) Water baths with shaker (Julabo SW23), Autoclave Sanyo;
2. Workstation for nucleic acids isolation: Laminar chamber (Holten 90), Centrifuges (Hettich Universal 32R, Eppendorf), Gel electrophoresis device (BIO-RAD PowerPac), UV Transilluminator (WEALTEC), Thermocycler (BIO-RAD MJ Mini), Whipper TissueRuptor Qiagen;
3. Western blot workstation: Polyacrylamide gel electrophoresis device (BIO-RAD PowerPac),

Semi-dry transfer system (TE70PWR Amer-sham Biosciences), Wet transfer system (BIO-RAD PowerPac), RollMixer Chemland, Protein detection system (SNAP i.d. Millipore);

4. Workstation for immunoenzymatic analyses (ELISA): microplate reader (KHB), Spectrophotometer working with cuvetes and microplates (Multiscan Spectrum, Thermo Scientific);
5. Workstation for immunoisotopic analyses (RIA): Beta and Gamma radiation counters, Beckman centrifuges;
6. Workstation for morphometric blood analysis: Hematological analyzer Advia 2120i;
7. Workstation for immunochemical blood analysis: ImmunoCAP 100;
8. Workstation for blood gasometry, oxymetry parameters, electrolytes and glucose analyses: Rapidpoint 405.



Selected papers

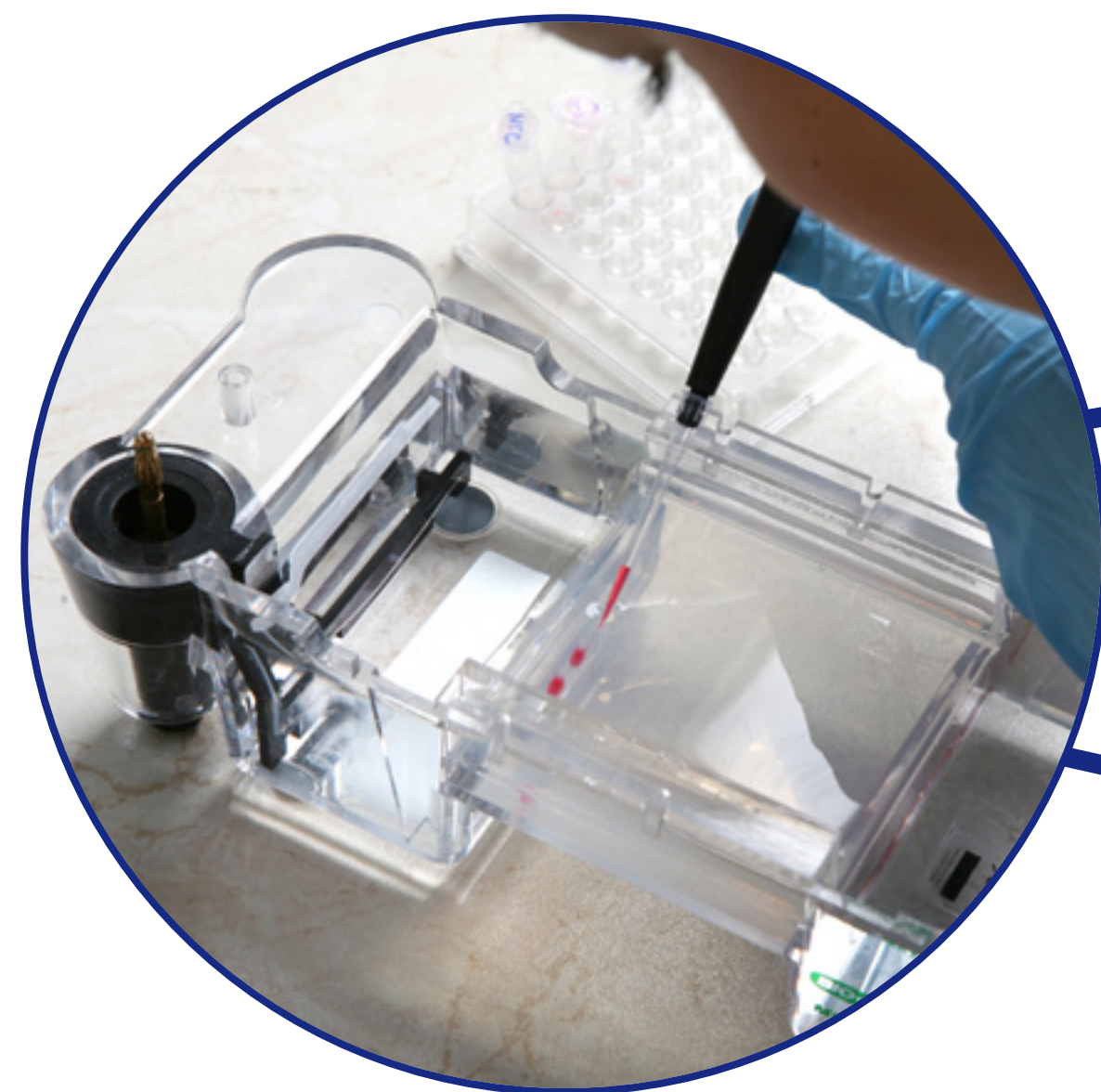
1. Galvao A.M., Henriques S., Pestka D., Lukasik K., Skarzynski D.J., Mateus L.M., Ferreira-Dias G.M. (2012) Equine luteal function regulation may depend on the interaction between cytokines and vascular endothelial growth factor. *Biology of Reproduction* 86 (6): 187, 1-9.
2. Majewska M., Lee H-Y., Tasaki Y., Acosta T.J., Szostek A.Z., Siemieniuch M., Okuda K., Skarzynski D.J. (2012) Is cortisol a modulator of interferon- α action in the endometrium during the early pregnancy in cattle? *Journal of Reproductive Immunology* 93: 82-9.
3. Kowalczyk-Zieba I., Izabela Woclawek-Potocka I., Piskula M.K., Piotrowska-Tomala K.K., Bah M.M., Siemieniuch M.J., Skarzynski

D.J. (2011) Experimentally induced mastitis and metritis modulate soy bean derived isoflavone biotransformation in dairy cows. *Theriogenology* 76: 1744-1755.

4. Jana B., Koszykowska M., Czarzasta J. (2011) Expression of nerve growth factor and its receptors, TrkA and p75, in porcine ovaries. *Journal of Reproduction and Development* 57: 468-474.
5. Koszykowska M., Calka J., Ganko M., Jana B. (2011) Long-term estradiol-17 β administration reduces population of neurons in the sympathetic chain ganglia supplying the ovary in adult gilts. *Experimental and Molecular Pathology* 91: 353-361.
6. Galvao A., Ramilo R., Skarzynski D.J., Lukasik K., Tramontano A., Mollo A., Mateus L., Ferreira-Dias G. (2010) Is Fas/Fas Ligand System Involved in Equine Corpus Luteum Functional Regression? *Biology of Reproduction* 83(6): 901-908.
7. Korzekwa A.J., Bah M.M., Kurzynowski A., Lukasik K., Groblewska A., Skarzynski D.J. (2010) Leukotrienes modulate secretion of progesterone and prostaglandins during the estrous cycle and early pregnancy in cattle: an in vivo study. *Reproduction* 140 (5): 767-776.
8. Majewska M., Woclawek-Potocka I., Bah M.M., Hapunik J., Piotrowska K.K., Tasaki Y., Acosta T.J., Okuda K., Skarzynski D.J. (2010) Is interleukin-1 α a luteotropic or luteolytic agent in cattle? *Reproduction* 139 (3): 665-672.
9. Siemieniuch M.J., Bowolaksono A., Skarzynski D.J., Okuda K. (2010) Ovarian steroids regulate prostaglandin secretion in the feline endometrium. *Animal Reproduction Sciences* 120(1-4): 142-150.

cytokines, prostaglandins, leukotrienes, lisophosphatic acid, corpus luteum, uterus, luteolysis, maternal recognition of pregnancy, embryo mortality, endometritis

Contact
Dariusz J. Skarzynski, Prof.
d.skarzynski@pan.olsztyn.pl



10. Woclawek-Potocka I., Komiyama J., Saulnier-Blache J.S., Brzezicka E., Bah M.M., Okuda K., Skarzynski D.J. (2009) Lysophosphatic acid modulates prostaglandin secretion

in the bovine uterus in vitro and in vivo. *Reproduction* 137 (1): 95-105.

Collaboration partners

- Laboratory of Reproductive Endocrinology, Graduate School of Natural Science and Technology, Okayama University, Japan;
- Department of Structure and Function, C.I.I.S.A., Faculty of Veterinary Medicine, Technical University in Lisbon, Portugal;
- Department of Obstetrics and Gynecology, C.I.I.S.A., Faculty of Veterinary Medicine, Technical University in Lisbon, Portugal;
- Department of Dairy and Animal Science, Center of Reproductive Biology and Health, Pennsylvania University, Pennsylvania, USA;
- Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel;
- Institute of Veterinary Anatomy, Vetsuisse Faculty of the University of Zurich, Switzerland.

Department of Physiology and Toxicology of Reproduction

Division of Reproductive Biology
Bydgoska 7 Str., 10-243 Olsztyn



Head
Jan Kotwica, Prof.

Assistant Professors
Robert Rękawiecki, Ph.D.
Jarosław Młynarczyk, DVM
Magdalena Duras, Ph.D.
Michał Wróbel, Ph.D.

Specialists
Beata Czarkowska, M.Sc.
Beata Ustjanowska, M.Sc.

Technician
Beata Jarmicka

Ph.D. Student
Anna Ziółowska, M.Sc.

M.Sc. Students
Paweł Będziechowski
Marlena Grzeszczyk

Focus areas

Studies carried out in the Department of Physiology and Toxicology of Reproduction involve molecular effect of steroids on the function of ovary and uterus in cows and genotoxic impact of environmental factors (xenobiotics) and non-nutritive feed components (phytoestrogens) on the function of reproductive tract. In particular, the research include: (1) regulation of steroidogenesis in corpus luteum (CL) during the estrous cycle and early pregnancy, including (a) participation of isoforms A (PRA) and B (PRB) of progesterone (P4) receptor and determination of the cDNA sequence for PRB, (b) mechanism of non-genomic effect of P4 on the cell function of bovine reproductive tract. These recent studies aim to determine (c) profile of expression for genes and proteins of P4 membrane receptors: PGRMC1, PGRMC2, SERBP1 and nuclear receptors (PR) in the endometrium and myometrium of the uterus and in the CL and also (d) cellular localization of PGRMC1, SERBP1 and PR proteins in the uterus and ovary. What is more, investigated is (2) the mechanism of the adverse impact of chloroorganic environmental contaminants on the: (a) secretory function of steroidogenic ovarian cells, (b) the influence of these compounds on the function of epithelial-chorionic connections in the bovine placenta, (c) secretory and contractory function in the bovine uterus and oviduct during the estrous cycle and the first trimester of pregnancy. The results of these studies may contribute to understanding the causes of impairments in the cattle reproduction at the molecular level.



Current research

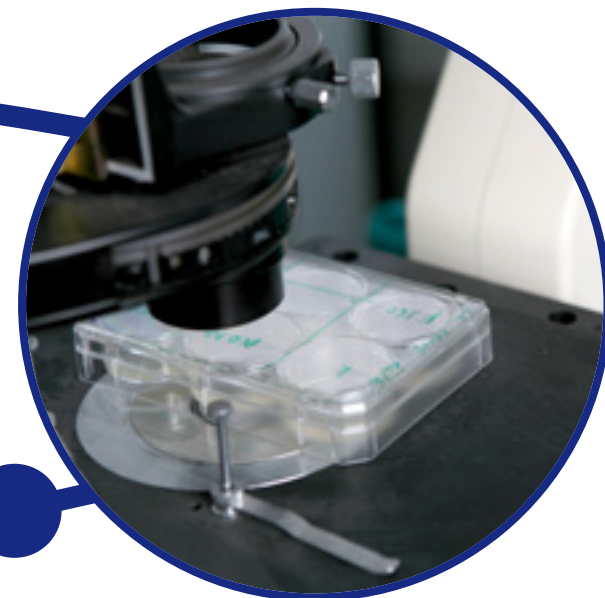
1. Validation of housekeeping genes for studying differential gene expression in bovine reproductive tract;
2. Molecular mechanism of xenobiotics effect on the function of bovine ovarian cells: involvement of orphan nuclear receptor SF-1 in PCBs and DDT-stimulated oxytocin synthesis/secretion;
3. Molecular mechanism of xenobiotics (PCBs and DDT) and the influence of their metabolites on the oviduct function in cattle;
4. Estimation of pesticides effect on the reproductive processes with the use of bovine reproductive tract as the experimental model;
5. Mechanism of non-genomic progesterone ef-

fect on the function of ovarian and uterine cells during estrus cycle and early pregnancy: involvement of progesterone membrane receptor, protein kinase G and A and B progesterone receptor isoforms.

The results obtained will provide data on the cellular and sub-cellular mechanism of reproductive tract functioning, using a cow as the experimental model under physiological conditions and under the influence of environmental contamination. This will contribute to expanding the knowledge on the mechanism of action of endogenous hormones, hormonal drugs and the impact of environmental changes on the reproductive processes of animals and humans.

Achievements

- demonstration of the expression of mRNA for membrane progesterone (P4) receptors (PGRMC1, PGRMC2 and SERBP1) in bovine uterus and corpus luteum (CL). Via these receptors P4 in non-genomic way can inhibit the secretion of uterine PGF2 α which is luteolytic, but not PGE2 which has luteotropic properties. This effect of P4, but also other steroids, may support of CL function and reduce the action of PGF2 α , and in this way exert influence on maintenance of early pregnancy;



- determination of the influence of luteotropic and luteolytic factors on the regulation of CL function at the molecular level (gene expression of the steroidogenesis; StAR, cytochrome P450scc, 3 β -HSD and receptors genes; PR-R and OT-R). Obtaining a partial cDNA sequence of isoform B of the PR;
- exhibition of the adverse molecular effect of polychlorinated xenobiotics on synthesis and secretion of PGF2 α and PGE2 in uterus and oviduct as well as oxytocin in ovarian steroidogenic cells. What is more, the genotoxic effect of the studied xenobiotics upon contractions of myometrium and muscle of oviduct in cow during estrous cycle was found.

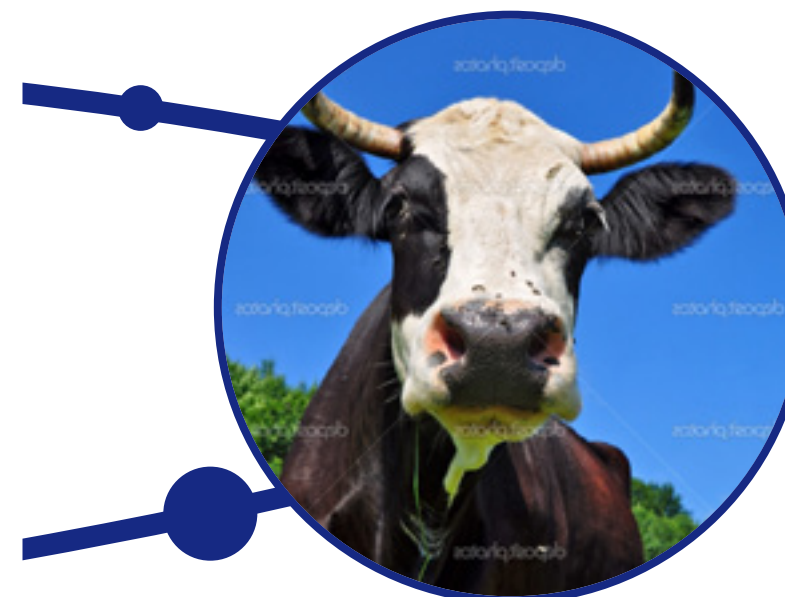
Selected papers

- Mlynarczyk J., Wrobel M., Kotwica J. (2011) The adverse effect of phytoestrogens on the secretory activity of bovine granulosa and luteal cells: involvement in oxytocin synthesis and secretion. *Reprod. Dom. Anim.* 46: 21-28.

- Rekawiecki R., Nowocin A., Kotwica J. (2010) Concentrations of progesterone, oxytocin and noradrenaline gene expression and protein level for their receptors in corpus luteum during estrous cycle in cows. *Prostaglandins Other Lipid Mediat.* 92: 13-18.
- Mlynarczyk J., Wrobel M.H., Kotwica J. (2010) Effect of environmental pollutants on progesterone and oxytocin secretion and on expression of genes involved in oxytocin synthesis in corpus luteum and on contractions of uterus in pregnant cows. *Toxicol. Appl. Pharmacol.* 247: 243-249.
- Wrobel M.H., Mlynarczyk J., Kotwica J. (2010) Increase of prostaglandins secretion from epithelium of bovine oviduct under influence of polychlorinated biphenyls and their metabolites, in vitro. *Toxicology* 270: 85-91.
- Slonina D., Kowalik M.K., Subocz M., Kotwica J. (2009) Genomic and non-genomic effect of ovarian steroids on oxytocin-stimulated secretion and synthesis of prostaglandins in bovine myometrial cells. *Prostaglandins Other Lipid Mediat.* 90: 69-75.
- Sirotkin, A.V., Rafay J., Kotwica J. (2009) Leptin controls rabbit ovarian function in vivo and in vitro: Possible interrelationships with ghrelin. *Theriogenology* 72: 765-772.
- Wrobel H.M., Rekawiecki R., Kotwica J. (2009) Involvement of prostaglandin F2 α in the adverse effect of PCB 77 on the force of contractions of bovine myometrium. *Toxicology* 262: 224-229.
- Sirotkin A.V., Rafay J., Kotwica J., Darlak K., Valenzuela F. (2009) Role of ghrelin in regulating rabbit ovarian function and the response to LH and IGF-I. *Dom. Anim. Endocrinol.* 36: 162-172.

**cattle, ovary, uterus,
progesterone, noradrenaline,
estrus cycle, endocrine
disruptors, phytoestrogens**

Contact
Jan Kotwica, Prof.
j.kotwica@pan.olsztyn.pl



- Mlynarczyk J., Wrobel M., Kotwica J. (2009) The influence of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolite-dichlorodiphenyldichloroethylene (DDE) on mRNA expression for NP-I/OT and PGA, involved in oxytocin synthesis in bovine granulosa and luteal cells. *Reprod. Toxicol.* 28: 354-358.
- Wrobel M., Mlynarczyk J., Kotwica J. (2009) The adverse effect of dihydrodiphenyltrichloroethane (DDT) and its metabolite (DDE) on the secretion of prostaglandins and oxytocin in bovine cultured ovarian and endometrial cells. *Reprod. Toxicol.* 27: 72-78.

Collaboration partners

- Research Institute of Animal Production, Nitra, Slovakia;
- Department of Animal Science, University of Kentucky, Lexington, USA;
- Banat's University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania.

Department of Local Physiological Regulations

Division of Reproductive Biology
Bydgoska 7 Str., 10-243 Olsztyn



Head

Stanisława Stefańczyk-Krzymowska, Prof.

Associated Professors

Janina Skipor, DVM, D.Sc.
Barbara Wąsowska, DVM, D.Sc.

Assistant Professor

Jolanta Muszak, DVM, Ph.D.

Specialist

Przemysław Gilun, M.Sc.

Technologist

Grażyna Rydelska

Senior Technicians

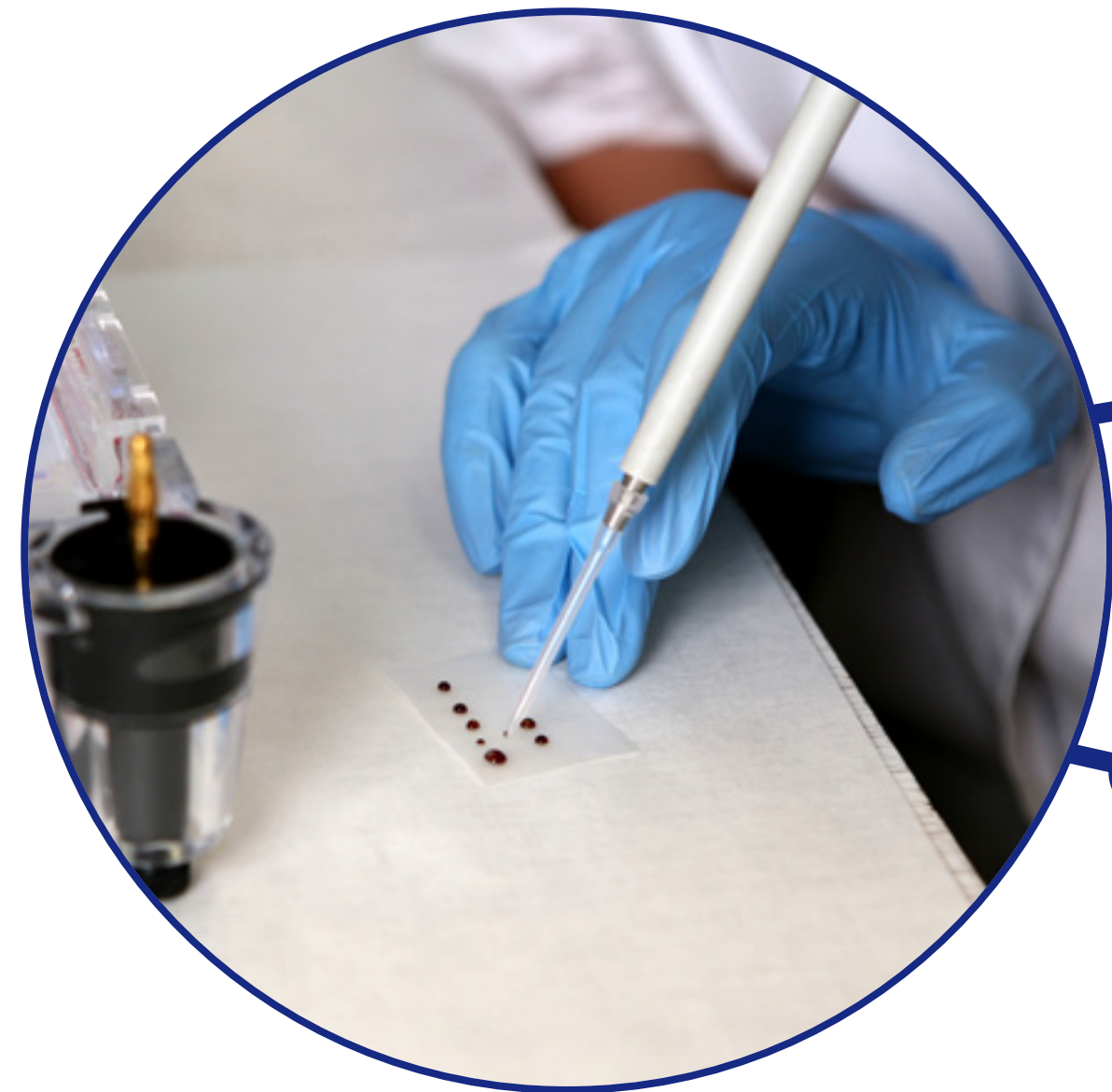
Joanna Winnicka
Zbigniew Strurzyński

Ph.D. students

Aleksandra Szczepkowska, M.Sc.
Joanna Kraszewska, M.Sc.

Focus areas

The aim of the research conducted in the Department of Local Physiological Regulations is to explain the role of central and local regulations in control of female reproductive organs and their mechanisms. Studies performed on the central level focus on the evaluation of the effect the environmental (photoperiod, polychlorinated biphenyls – PCB) and nutritional (phytoestrogens) factors exert on the access of regulatory substances to the hypothalamus, as well as the role cerebrospinal fluid (CSF) and choroid plexus play in mechanisms regulating these processes. Molecules present in the CSF may reach the hypothalamus and then modulate cell activity of several hypothalamic neuronal populations expressing neuromodulators important for both the central control of reproduction and food intake. The subject of research concerns bioavailability of PCB and phytoestrogens in the CSF and the influence of environmental factors on integrity of blood-CSF barrier, localized in the choroid plexuses, as well as on protein profile of CSF. Studies are conducted in collaboration with Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences and French National Institute for Agricultural Research. Research on the local regulation of reproductive organs focus on the role of vasculature in the regulation of the ovary and uterus function. Earlier studies at the Department demonstrated that blood and lymphatic vessels of the mesovarium create the system in which hormones released from the ovary permeate to arterial blood by a counter current mechanism and are retrogradely transferred into the ovary. It was also revealed that adjustment of the broad ligament vasculature provides



additional supply of the uterus with ovarian hormones by local destination transfer. Ongoing studies concern the influence of the ovary supply with elevated blood concentration of ovarian hormones on its secretory function, and the effect of local supply of the uterus with ovarian hormones, including changes in uterine blood supply regulated by ovarian hormones, on cyclic functions of the endometrium.

Current research

On central level:

1. Bioavailability of phytoestrogens, their metabolites and PCB in the CSF, and their effect on neuroendocrine mechanisms regulating seasonality of reproduction in ewes;
2. Effect of photoperiod and PCB on the expression of tight junction proteins, and the vas-

cular endothelial growth factor (VEGF) with its receptors in the choroid plexuses in ewes - *in vivo* study;

3. Proteomic analysis of protein profile in CSF;
4. Influence of melatonin on the response of the choroid plexuses of the brain on the immunological stress.

On local level:

5. Expression of Hypoxia Inducible Factor (HIF1α) in the uterus during the estrous cycle in the pig.

The objective of the studies is to test the relationship between variable uterine blood supply and the level of expression of HIF1α, stimulating the transcription of genes responsible for tissue oxygen homeostasis, and to determine the influence of the uterine



horn local supply with ovarian hormones on HIF1 α expression.

6. Energy status and location of antioxidant enzymes in the uterus under variable blood supply conditions.

Research aims at estimating the endometrium energy charge and the concentration of purine metabolism products under conditions of physiological and experimental hypoxia, and determination of the activity of antioxidant enzymes in the endometrium during its cyclic rebuilding.

Achievements

- demonstrating that photoperiod modulates bioavailability of PCB153 in sheep, what may be connected with seasonal changes of adipose tissue activity in sheep;
- elucidating that choroid plexus "plasticity" in ewes, including the effect of photoperiod on the expression of tight junction proteins and VEGF system;
- revealing the inhibitory effect of PCB153 on tight junctions expression in the choroid plexus, what suggests that PCB153 may increase the permeability of blood-cerebrospinal fluid barrier;
- demonstrating that retrograde transfer of progesterone into the ovary during luteal phase stimulates its own secretion (local feedback) and secretion of androstenedione and activin A, but decreases estrogens secretion;
- showing that releases of prostaglandin F2 α concentration in uterine venous blood occurring after luteolysis are mainly the results of

the excretion taking place due to contractions of the uterus caused by oxytocin;

- exhibiting a significant level of HIF-1 α expression, modulated by local ovarian hormones supply, in the porcine endometrium during reduced uterine blood supply in mid- and late-luteal phase of the estrous cycle.

Analytical facilities:

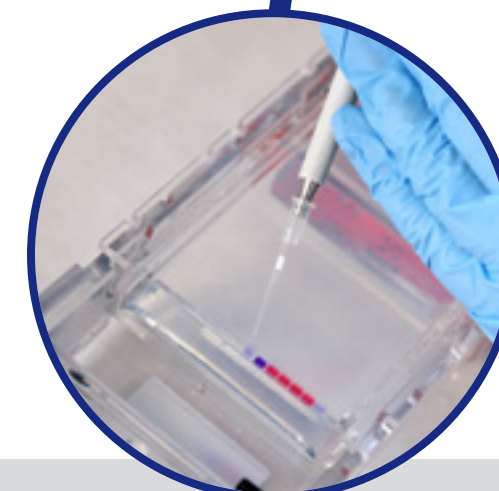
1. Perfusion system for study on isolated organs (UNIPER UP-100; Hugo Sachs Elektronik-Harvard);
2. System for the study on isolated blood vessels (HSE F30, type 372; Hugo Sachs Elektronik);
3. Beta counter (Liquid scintillation spectroscopy, LS 500 TD Beckman Instruments);
4. Gamma counter (LKB Wallac-Clinigamma);
5. Infuse/Withdraw Pump (PHD 2000, Hugo Sachs Elektronik Harvard Apparatus, Germany).

Selected papers

1. Koziorowski M., Stefanczyk-Krzymowska S., Tabecka-Lonczynska A., Gilun P., Kaminski M. (2012) Gaseous messenger carbon monoxide is released from the eye into the ophthalmic venous blood depending on the intensity of sunlight. *Journal of Biological Regulators and Homeostatic Agents* 26: 111-118.
2. Krzymowski T., Stefanczyk-Krzymowska S. (2012) Local retrograde and destination transfer of physiological regulators as an important regulatory system and its role – facts and hypothesis. *Journal of Physiology and Pharmacology* 63: 1-14.
3. Skipor J., Mlynarczuk J.J., Szczepkowska A., Lagaraine C., Grochowalski A.,

Guillaume D., Dufourny L., Thiéry J-C. (2012) Photoperiod modulates access of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) to the brain and its effect on gonadotropin and thyroid hormones in adult ewes. *Ecotoxicology and Environmental Safety* 78: 336:343.

4. Stefanczyk-Krzymowska S., Wasowska B., Gilun P., Muszak J., Grzegorzewski W. (2012) Relationship between contractions of the uterus and concentration of PGF2 α in uterine venous blood after luteolysis in gilts. *Reproduction in Domestic Animals* 47: 98-104.
5. Skipor J., Misztal T., Piskuła M., Wiczkowski W., Thiery J-C. (2012) Phytoestrogens and thyroid hormone levels in the cerebrospinal fluid of ewes fed red clover silage. *Small Ruminants Research* 102:157-162.
6. Jablonska O., Piasecka J., Petroff B.K., Nynca A., Siawrys G., Wasowska B., Zmijewska A., Lewczuk B., Ciereszko R.E. (2011) In vitro effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on ovarian, pituitary, and pineal function in pigs. *Theriogenology* 76: 921-932.
7. Lagaraine C., Skipor J., Szczepkowska A., Dufourny L., Thiéry J-C. (2011) Expression of tight junction proteins is different in choroid plexus of ewes according to photoperiod. *Brain Research* 1393: 44-51.
8. Skipor J., Misztal T., Kaczmarek M.M. (2010) Independent changes of thyroid hormones in blood plasma and cerebrospinal fluid after melatonin treatment in ewes. *Theriogenology* 74: 236-245.
9. Grzegorzewski W., Chlopek J., Tabecka-Lonczynska A., Stefanczyk-Krzymowska S. (2010) The influence of steroids on vascular tension of isolated superficial veins of the nose and face during the estrous cycle of gilts. *Theriogenology* 73: 215-224.
10. Wasowska B., Stefanczyk-Krzymowska S. (2009) The effect of unilateral progesterone infusion into ovarian artery during the middle luteal phase on progesterone secretion in conscious gilts. *Reproductive Biology* 9: 73-86.



**photoperiod,
tight junction proteins,
choroid plexus, cerebrospinal
fluid, polychlorinated biphenyls,
steroid hormones, prostaglandins,
local feedback, Hypoxia Inducible
Factor HIF-1 α , energy status**

Contact
Stanisława Stefanczyk-
Krzymowska, Prof.
s. stefanczyk-krzymowska@
pan.olsztyn.pl

Collaboration partners

- Physiologie de la Reproduction et des Comportements, Inra-Crns-Universite F. Rabelais, Nouzilly, France;
- Heart Science Centre, Imperial College at Harefield Hospital, Great Britain.

Department of Gamete and Embryo Biology

Division of Reproductive Biology
Bydgoska 7 Str., 10-243 Olsztyn



Head
Andrzej Ciereszko, Prof.

EMBRYO BIOLOGY TEAM

Marek Bogacki, Ph.D.
Assistant Professors
Beenu Moza Jalali, Ph.D.
Teresa Więsak, Ph.D.
Assistant
Marta Wasielak, Ph.D.
Senior Technologist
Michał Blitek, M.Sc.
Specialist
Małgorzata Karasim, M.Sc.
Ph.D. Student
Anna Kitewska, M.Sc.

SEMEN BIOLOGY TEAM

Assistant Professors
Mariola Dietrich, Ph.D.
Mariola Słowińska, Ph.D.
Grzegorz Janusz Dietrich, Ph.D.
Joanna Nynca, Ph.D.
Senior Specialist
Halina Karol, Eng.
Technologists
Ewa Liszewska, M.Sc.

MOLECULAR ANDROLOGY TEAM

Professor
Jan Glogowski, Prof.
Associated Professor
Zygmunt Giżejowski, Ph.D., D.Sc.
Assistant Professors
Beata Sarosiek, Ph.D.
Radosław Kajetan Kowalski, Ph.D.
Assistant
Beata Irena Cejko, Ph.D.
Senior Specialist
Wiesław Jerzy Demianowicz, DVM, Ph.D.
Technologist
Katarzyna Dryl, M.Sc.

Focus areas

Scientific scope of the research conducted in the Department of Gamete and Embryo Biology ranges from biochemistry and physiology of animal spermatozoa to practical aspects of fish reproduction. The area of research comprises also the issues related to the embryonic development in pre- and peri-implantation period and oocytes quality in regard to the future embryo development. The studies being performed aim at the determination of the influence of assisted reproductive techniques on oocytes and embryos quality, as well as the role of female genes in the regulation of oocytes maturation and early embryo development. The scientific investigations concentrate also on the interactions between the embryo and uterine structures during early pregnancy, with a special emphasis put on the maternal immunotolerance processes for a developing embryo. Biochemical research conducted at the Department focus on the isolation and characterization of proteins and peptides of animal seminal plasma and spermatozoa, with the use of classical and proteomic methods. Research on physiology involves studies of sperm motility with the use of computer-assisted sperm analysis (CASA) and analysis of sperm DNA damage using a comet assay. Applied research concerns the perfection of short and long term storage of fish and avian semen, foundation of the Fish Sperm Bank, and estimation of egg quality of salmonid fish.

Current research

1. Isolation, identification and characterization of proteins of fish semen, such as transferrin, parvalbumin, apolipoprotein and serine pro-



teinases and their inhibitors in fish and avian semen;

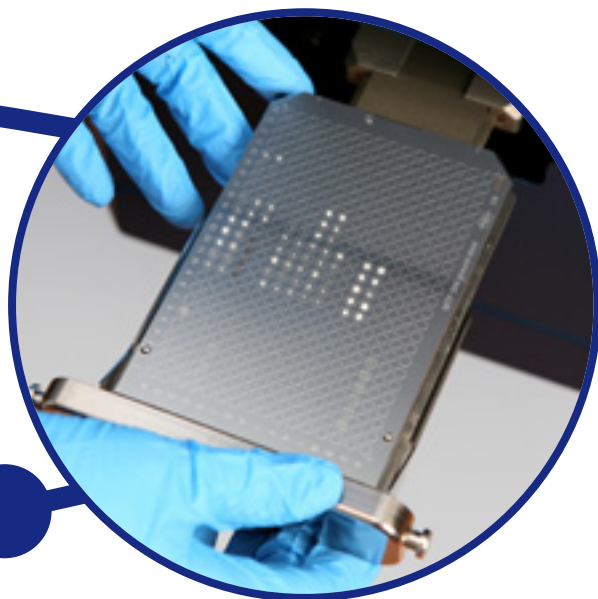
2. Proteomic analysis of rainbow trout and common carp seminal plasma;
3. Cryopreservation of fish semen;
4. Determination of swine oocytes and embryos quality obtained from animals after different methods of estrous stimulation;
5. Investigation of embryo influence on maternal immune system for increasing the immunotolerance to a developing embryo.

The aim of the research conducted is to broaden the knowledge associated with fish, avian and mammal reproduction and, in particular, with the role of seminal plasma proteins in the male reproductive system. The scientific objectives realized by the research staff of the Department are also

concerned with the characteristics of oocytes and their quality within the female reproductive processes. This knowledge may have a crucial importance when it comes to improving the methods of animal reproduction, for instance in relation to the improvement of short and long term semen cryopreservation, as well as the investigation of semen and oocytes quality determinants. Demonstration of the relation between specific seminal proteins and their quality will contribute to the development of quick sperm quality evaluation methods.

Achievements

- identification and characterization of fish semen proteins: α_1 -antiproteinase, fetuin-B-like protein; lipocalin-type protein; apolipoproteins C-I from rainbow trout seminal plasma



and α_1 -antiproteinase, transferrin, parvalbumin from common carp seminal plasma;

- improvement of breeding and reproduction methods of black and wood grouse to determine chances for introduction of these birds to the environment without a loss of their biodiversity. Black and wood grouse semen characteristics have been obtained for the first time;
- isolation and biochemical characteristics of arylsulphatase from Siberian sturgeon semen. Isolation and characterization of arylsulphatase and acid phosphatase from spermatozoa of rainbow trout have been conducted for the first time;
- elaboration of *in vivo* research model for investigation of embryo and uterus interactions in early stages of pregnancy, and determination of hormonal profiles typical of this period in pigs;
- demonstration of the anti-apoptotic effect of IGF-1 during bovine oocytes maturation and pig embryos development *in vitro*.

Analytical facilities

1. Vertical electrophoresis system and apparatus for transfer;
2. Apparatus for measuring sperm concentration and viability (NucleoCounter SP-100);
3. High-performance liquid chromatography system (HPLC) and fast protein liquid chromatography system (FPLC);
4. Integrated system for the comet assay (horizontal electrophoresis system, fluorescence microscope);
5. Systems for computer analysis of sperm mo-

tion (CASA) - Hobson's Vision (analog-digital system);

6. Computer controlled system for freezing the biological material.

Selected papers

1. Cejko B.I., Kowalski R.K., Kucharczyk D., Targonska K., Krejszeff S., Zarski D., Glogowski J. (2010) Influence of the length of time after hormonal stimulation on selected parameters of milt of ide *Leuciscus idus* L. *Aquaculture Research* 41: 804-813.
2. Ciereszko A., Rybnik P. K., Horbanczuk J.O., Dietrich G.J., Deas A., Slowinska M., Liszewska E., Malecki I.A. (2010) Biochemical characterization and sperm motility parameters of ostrich (*Struthio camelus*) semen. *Animal Reproduction Science* 122: 222-228.
3. Dietrich M.A., Zmijewski D., Karol H., Hejmej A., Bilinska B., Jurecka P., Irnazarow I., Slowinska M., Hliwa P., Ciereszko A. (2010) Isolation and characterization of transferrin from common carp (*Cyprinus carpio* L) seminal plasma. *Fish & Shellfish Immunology* 29: 66-74.
4. Dietrich G.J., Dietrich M., Kowalski R.K., Dobosz S., Karol H., Demianowicz W., Glogowski J. (2010) Exposure of rainbow trout milt to mercury and cadmium alters sperm motility parameters and reproductive success. *Aquatic Toxicology* 97: 277-284.
5. Franczak A., Bogacki M. (2009) Local and systemic effects of embryos on uterine tissues during early pregnancy in pigs. *Journal Reproduction and Development* 55 (3): 262-272.

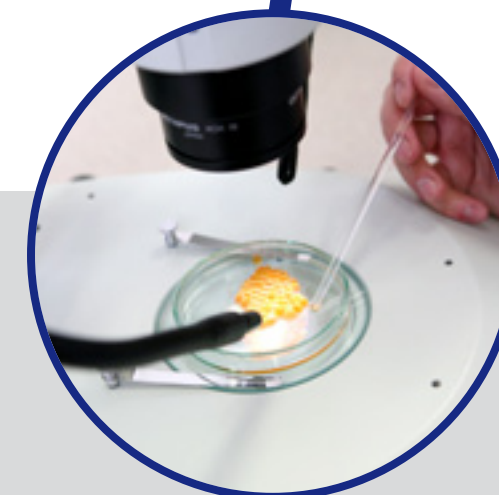
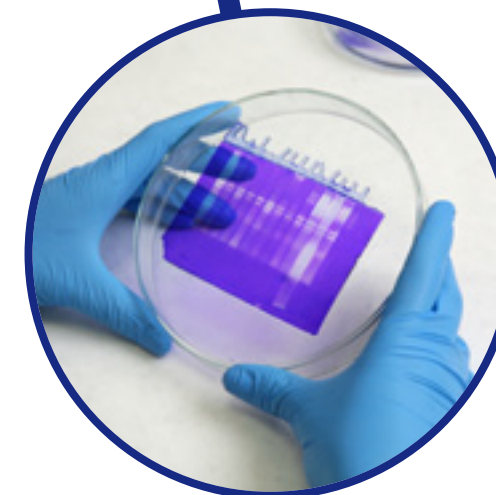
**seminal plasma,
sperm, cryopreservation,
proteinases, inhibitors,
embryo, oocyte**

Contact
Beata Irena Cejko, Ph.D.
b.cejko@pan.olsztyn.pl

6. Gizejewski Z., Söderquist L., Rodriguez Martinez H. (2010) Genital and sperm characteristics of wild, free ranging red deer stags (*Cervus elaphus* L) hunted in different regions of Poland. *Wildlife Biology in Practice* 6 (2): 81-94.
7. Kowalski R.K., Sarosiek B., Demianowicz W., Judek J., Goryczko K., Dobosz S., Kuzmiński H., Demska-Zakes K., Babiak I., Glogowski J. (2011) Quantitative characteristics of rainbow trout, *Oncorhynchus mykiss*, neo-males (XX genotype) and super-males (YY genotype) sperm. *World Academy of Sciences, Engineering and Technology* 77: 1162-1169.
8. Nynca J., Dietrich M.A., Bilinska B., Kotula-Balak M., Kielbasa T., Karol H., Ciereszko A. (2011) Isolation of lipocalin-type protein from rainbow trout seminal plasma and its localization in the reproductive system. *Reproduction, Fertility and Development* 23: 381-389.
9. Slowinska M., Olczak M., Liszewska E., Watorek W., Ciereszko A. (2010) Isolation, characterization and cDNA sequencing of acrosin from turkey spermatozoa. *Comp Biochem Physiol B Biochem Mol Biol*. 157: 127-136.
10. Wasielek M., Kaminska K., Bogacki M. (2009) Effect of the conceptus on uterine prostaglandin-F2alpha and prostaglandin-E2 release and synthesis during the periimplantation period in the pig. *Reproduction, Fertility Development* 21 (5): 709-717.

Collaboration partners

- The Ohio State University, Columbus, USA;
- South Bohemia University, Vodnany, Czech Republic;
- University of Paris 6, Villefranche sur mer, France;
- Bodo University College, Norway;
- Canary Institute of Marine Sciences, Las Palmas, Spain;
- Saint Istvan University, Godollo, Hungary;
- Russia Research Institute for Hunting VNIIOZ, RASS, Kiev, Russia;
- Iwate University, Faculty of Agriculture, Morioka, Japan.



Department of Biology and Pathology of Human Reproduction

Division of Reproductive Biology
24a M.C. Skłodowskiej Str., 15-274 Białystok



Head
Jan Czerniecki, Ph.D.

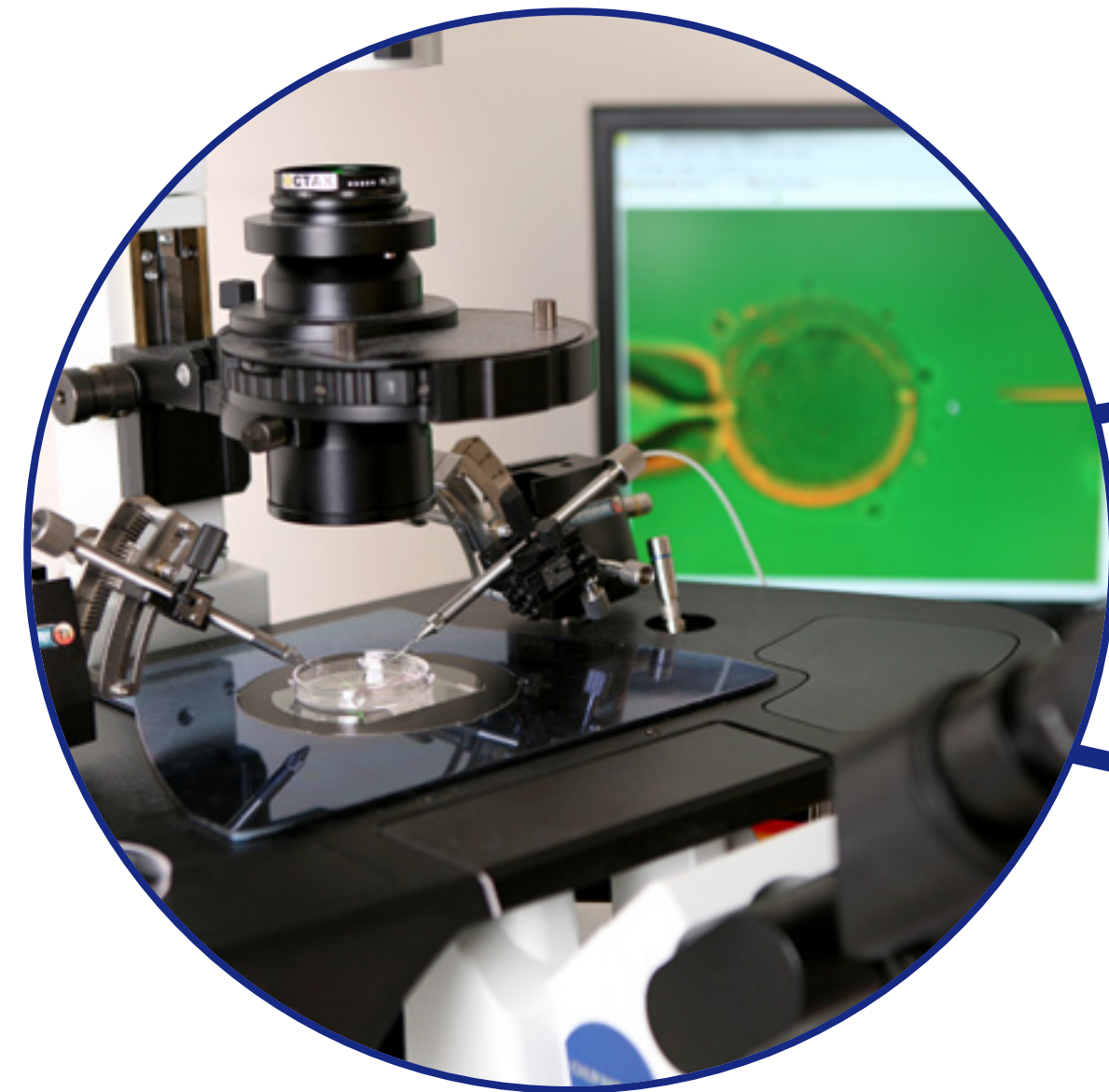
Professor
Sławomir Wołczyński, Prof.

Assistant Professor
Katarzyna Jarząbek, Ph.D.

Technologists
Tomasz Bielawski, Ph.D.
Donata Ponikwicka-Tyszko, M.Sc.
Maria Sztachelska, M.Sc.

Focus areas

The *in vitro* fertilization and embryo transfer is very often the only way of treatment of many infertile couples. Despite a significant development in human assisted reproductive technologies that has been made in recent years, many issues still remain unsolved. Difficulties encountered in assessing the reproductive and developmental potential of gametes make it necessary to fertilize more oocytes and to transfer at least two embryos into the uterus. This has a crucial impact on the effectiveness of treatment and leads to the increase in the percentage of multiple pregnancies and gynaecological complications associated with them. Therefore, it is important to develop new noninvasive methods of gametes quality and selection evaluation in order to improve the effectiveness of infertility treatment and to reduce the number of fertilized oocytes and embryos transferred into uterus. What is more, recent years show a constant tendency to decline in the quantitative and qualitative parameters of sperm: the sperm count, impairment of its movement and increase in the percentage of pathological forms. There are some implications suggesting that male infertility is affected by some chemical compounds present in everyday products, such as packages, clothes, furniture, carpeting and medical products- tooth fillings and contact lenses. As a result of their ability to interfere the action of natural hormones regulating reproduction processes, they are called endocrine disruptors (ECDs). An extremely important and urgent need is to identify factors responsible for the occurrence of male infertility, as well as to develop effective methods of prevention and reduction of their negative impact.



Current research

1. Evaluation of ovarian reserve and its impact on the reproductive potential of oocytes with an attempt to determine new biomarkers of their developmental quality;
2. Analysis of the influence of endocrine disruptors on sperm parameters and sperm fertilizing capacity;
3. Attempts to develop new methods of assessing the development ability and implantation potential of embryos;
4. Analysis of the endometrial receptivity.

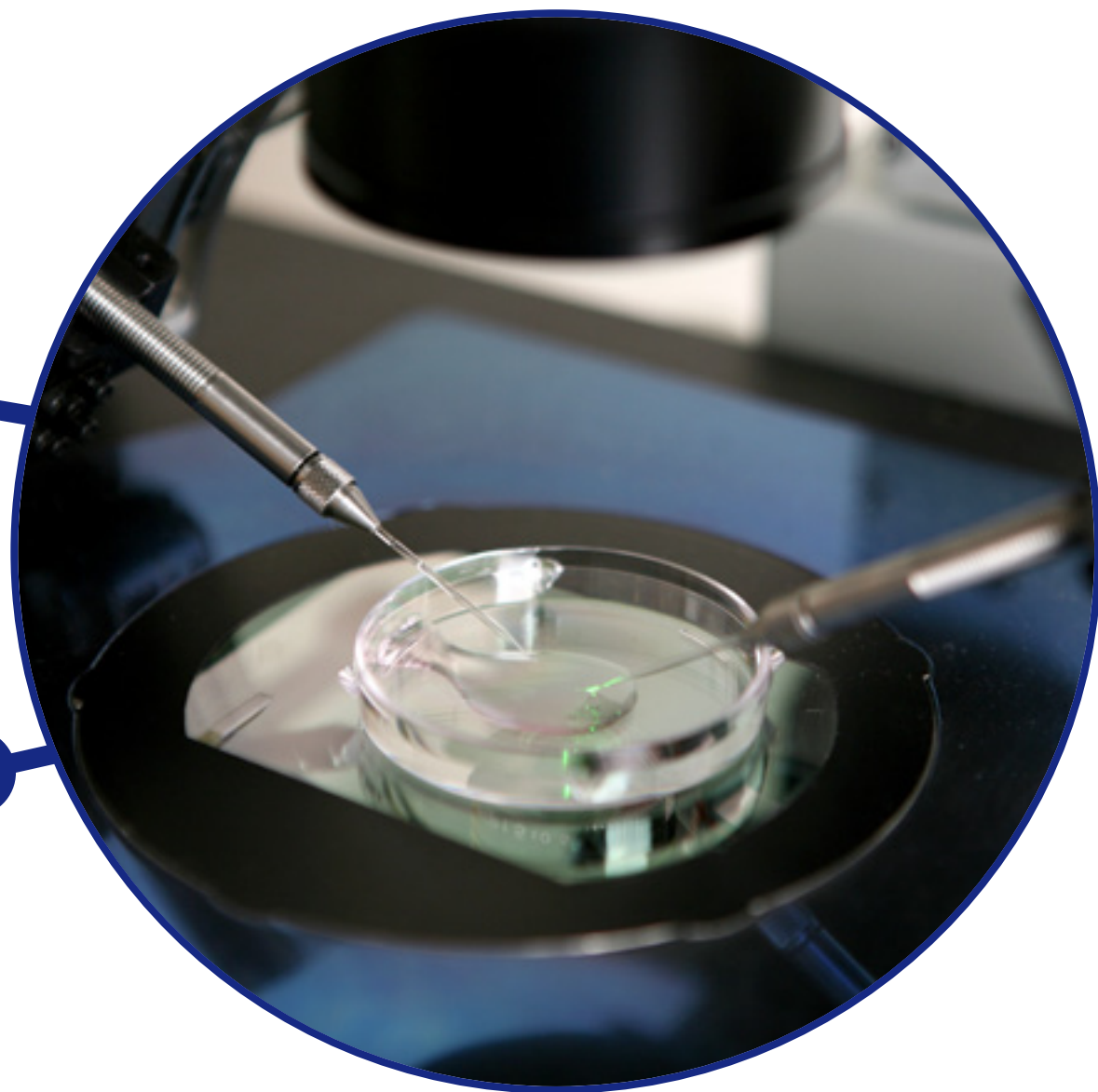
Analytical facilities

- Sperm Class Analyzer SCA-PACK-H-02 SCA Complete Human Pack: Sperm Class Analyzer Motility & Concentration, SCA Viewer, SCA Morphology, SCA Manual Counter – Microptic;

- Automatic station for nucleic acids isolation 6100 Nucleic Acid PrepStatus - Applied Biosystems;
- Real-Time PCR - Applied Biosystems;
- Sequencer 3130 Genetic Analyzer with analytical software - Applied Biosystems;
- MiniVE Vertical Electrophoresis and Blotting System, Uvitec system for gel analysis - Amersham;
- Laminair chamber K-System HV 2448, CO2 incubator - Heraeus, inverted microscope Eclipse TE2005 NIKON, cell freezing equipment Kryo 360-17 Plawer.

Selected papers

1. Ponikwicka-Tyszko D., Kotula-Balak M., Jarzabek K., Bilinska B., Wolczynski S., (2012) The DAX1 mutation in a patient with



hypogonadotropic hypogonadism and adrenal hypoplasia congenita causes functional disruption of induction of spermatogenesis. Journal of Assisted Reproduction and Genetics DOI 10.1007/s10815-012-9778-y.

2. Kida E., Walus M., Jarzabek K., Palminiello S., Albertini G., Rabe A., Hwang Y.W., Golabek A.A. (2011) Form of dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A nonphosphorylated at tyrosine 145 and 147 is enriched in the nuclei of astroglial cells, adult hippocampal progenitors, and some cholinergic axon terminals. Neuroscience 195: 112-27.
3. Czerniecki J., Wolczynski S., 2011, Deep sequencing - a new method and new requirements. Studies in Logic, Grammar and Rhetoric 25 (38): 41-48.

***assisted reproductive technologies,
in vitro fertilization, oocyte quality, sperm parameters, endocrine disruptors, gametes quality biomarkers***

Contact
Jan Czerniecki, Ph.D.
j.czerniecki@pan.olsztyn.pl

4. Milewski R., Malinowski P., Milewska A.J., Czerniecki J., Ziniewicz P., Wolczynski S. (2011) Nearest neighbor concept in study of IVF ICSI/ET treatment effectiveness. Studies in Logic, Grammar and Rhetoric 25 (38): 49-58.
5. Milewski R., Milewska A.J., Czerniecki J.,

Jamiolkowski J., Domitrz J., Wolczynski J. (2010) The statistical module for the system of electronic registration of information about patients treated for infertility using IVF ICSI/ET method. Studies in Logic, Grammar and Rhetoric 21 (34).

Collaboration partners

- Institut de Génétique et Développement de Rennes, France;
- Département de Biochimie, Université Caen, France;
- Institut National de la Santé et de la Recherche Médicale et Paediatric Endocrinologie, Département de Genetique et Reproduction Humaine, Paris V University, Caen, France;
- Department of Physiology, Institute of Biomedicine; University of Turku, Finland.

Division of Food Sciences

Department of Immunology and Food Microbiology

Division of Food Sciences
10 Tuwima Str., 10-748 Olsztyn



Head

Barbara Wróblewska, Ph.D., D.Sc.

Professors

Henryk Kostyra, Prof.
Lucjan Jędrychowski, Prof.

Assistant Professors

Lidia Markiewicz, Ph.D.
Dominika Świątecka, Ph.D.

Assistants

Ewa Wasilewska, Ph.D.
Dagmara Złotkowska, Ph.D.

Specialist

Małgorzata Panasiuk, M.Sc.

Technologist

Mirostaw Obrębski, M.Sc.

Ph.D. students

Anna Kaliszewska, M.Sc.
Justyna Chudzik-Kozłowska, M.Sc.
Anna Ogrodowczyk, M.Sc.

Focus areas

Scientific investigations carried out in the Department of Immunology and Food Microbiology are focused on the examination of the influence of food components on the immunological gastrointestinal tract and intestinal microbiota of the host. The main research directions are concerned with 1) the analysis of allergenicity of food components together with designing a technology that would reduce its activity, 2) the profile and activity of microbiota, and 3) interactions of intestinal bacteria with the organism of the host and food components.

The objectives realized in the Department include:

- analysis of raw food products, their selected components, as well as commercial and new, personalized food in the aspect of its immunogenic and allergenic properties;
- reducing allergenicity of food in the process of thermal, enzymatic, chemical modifications and bioconversion;
- study of interactions between food proteins with non-protein components under conditions similar to the technological processes, with a special emphasis put on the changes occurring in the functioning of the immune system triggered by products of the non-enzymatic glycosylation;
- evaluation of the influence of glycated proteins and peptides on human microbiota;
- improvement in host's tolerance as a result of oral immunotherapy - research conducted



on the animal model (BALB/c mouse) with induced food allergy;

- acquisition of probiotic strains with defined pro-health properties;
- analysis of the influence of dietary components on the functioning of human and animal gastrointestinal tract and the physiological balance of intestinal microbiota;
- defining new parameters of the properties of probiotic bacteria, such as the phytase activity.

Current research

1. "The effect of fermented milk drinks with reduced antigenicity on the immunological response of patients with food allergy in the Warmia and Mazury Province, including genetic determinants"
 - setting up the bank of sera obtained from in-

dividuals with allergies, constituting an electronic database on the allergy development in the region of Warmia and Mazury;

- determination of the presence of selected groups of intestinal bacteria in patients with food allergy in order to regulate their deficit through application of directed fermented milk products, enriched with selected strains;
 - analysis of mutation in the filaggrin gene as a novel marker pointing at genetic causes of food allergy.
2. "Influence of hydrolysis and non-enzymatic glycosylation on the induction of regulatory cells"
 - induction of regulatory cells by controlled administration of dietary proteins (hydrolysed or glycated) to stimulate the immunological response of the organism

- of the host and induce the state of tolerance.
3. "Phytase activity as a novel parameter of probiotic strains"
 - characterization of the intestinal bacteria in the context of their ability to decompose the phytic acid in individuals on conventional and vegetarian diet;
 - isolation, selection and characterization of strains with high phytase activity and properties reducing the immune-reactivity of soy bean proteins.



4. "Enzymatic hydrolysates of oat and rice proteins present in milk substitute drinks as factors modulating the physiological activity of microbiota and intestinal epithelium"
 - determination of the influence of peptides and glycopeptides released from food proteins on quality and quantity changes in the human gastrointestinal tract ecosystem;
 - designing new milk substitute formula with the use of plant proteins or their hydrolysates with defined biological properties.
5. "Evaluation of safety and pro-health activity of home and commercial probiotic preparations: model research on animals with induced colitis"
 - analysis of risks and benefits resulting from the consumption of probiotic preparations containing Bifidobacterium strains from home collection, exhibiting anticancer properties- research on the animal model with induced colitis.
6. "Whey protein hydrolysates as potential modulators of the intestinal ecosystem in the aspect of obesity"

- evaluation of the influence of whey protein hydrolysates on the profile and activity of intestinal microbiota in the context of obesity.

Achievements

- reduction of immunoreactivity and allergenicity of milk proteins in fermented drinks;
- modification of the technology of producing fermented milk drinks by the application of microbial transglutaminase for the reduction of allergenic potential, as a proposition of an alternative diet for individuals allergic to cow's milk protein;
- development of a technological procedure of the use of hydrolysed pea proteins on a murine model as a method of oral allergen immunotherapy in case of strong allergic hypersensitivity to peanuts;
- the use of dietary proteins, on the example of glycosylated and hydrolysed ovalbumin, for the induction of regulatory cells of a mouse immune system;
- elucidating in model animal studies the pro-health and anticarcinogenic activity of Bifidobacterium strains isolated from a human;
- isolation, characterization and identification of more than 600 strains of lactic acid bacteria, from home collection of the Institute;
- documentation of the influence of the diet on the activity of intestinal bacteria phytase, and elucidation of the activity of bacteria in the process of a total hydrolysis phytic acid.

Analytical facilities

1. Flow cytometer - BD LSRFortessa™ Cell Analyzer;

gastrointestinal immunology, allergy, allergenic food proteins, food tolerance, regulatory cells, non-enzymatic glycolysation of proteins, intestinal microbiota, probiotics

Contact
Barbara Wróblewska, Prof.
b.wroblewska@pan.olsztyn.pl

2. Cell and tissue culture laboratory equipment (laminar cabinet - BIOHAZARD, CO2 incubators, microscopes);
3. Chromatographic systems - HPLC system (Shimadzu), FPLC system (Pharmacia LKB) and AKTApurifier plus system (GE Healthcare);
4. Horizontal and vertical DNA electrophoresis systems (DCode System for DGGE - universal mutation detection system) as well one and two dimensional protein electrophoresis system - PROTEAN IEF Cell (Bio-Rad), Ettan IP-Gphor 3 (GE Healthcare);
5. Standard thermocyclers (Eppendorf, Bio-Rad) and real-time PCR system (iQ5, Bio-Rad);
6. Software for data analysis - Bionumerics (Applied Maths) and software for gel analysis - PDQuest Basic 2-D Analysis Software (Bio-Rad);
7. Laboratory equipment for immunometric determination by ELISA and ELISPOT methods as well ImmunoCAP analysis (specific Ig-E analysis and panel allergen determination in blood serum);
8. Workstations for anaerobic and aerobic intestinal microbiota examinations including: MG500 Anaerobic Workstation (Don Whitley Scientific, UK), multistage gastrointestinal model as well Biostat C bioreactor (Biotech International).

Selected papers

1. Wroblewska B., Kaliszewska A., Kolakowski P., Pawlikowska K., Troszynska A. (2011) Impact of transglutaminase reaction on the immunoreactive and sensory quality of yoghurt starter. World J. Microbiol. Biotechnol. 27 (2): 215-227.
2. Wroblewska B., Kaliszewska A. (2011) Immunoreactive and allergenic properties of fermented milk products present on the polish market. Milchwissenschaft 3: 300-303.
3. Swiatecka D., Narbad A., Ridgway K., Kostyra H. (2011) The Study on the Impact of Glycosylated Pea Proteins on Human Intestinal Bacteria. International Journal of Food Microbiology 145: 267-272.
4. Markiewicz L.H., Biedrzycka E., Wasilewska E., Bielecka M. (2010) Rapid molecular identification and characteristics of Lactobacillus strains. Folia Microbiol. 55 (5): 481-488.
5. Swiatecka D., Swiatecki A., Kostyra H., Marciniak-Darmochwał K., Kostyra E. (2010) The Impact of Pea Proteins Hydrolysates On Bacterial Physiological Activity - An In Vitro Study. International Journal of Food Microbiology 140: 263-270.
6. Swiatecka D., Swiatecki A., Kostyra H. (2010) Impact of the Glycosylated Pea Proteins on the Activity of Free-Swimming and Immobilized Bacteria. Journal of the Science of the Food & Agriculture 90 (11): 1837-45.
7. Zlotkowska D., Kozłowska J., Rudnicka B., Kostyra H. (2010) Immune responses of mouse mucosal system to glycosylated and hydrolysed OVA. FASEB J. 24: 723.719.
8. "Chemical and Biological Properties of Food Allergens". Series: "Chemical and Functional Properties of Food Components". Edited by Lucjan Jędrychowski, Harry J. Wichers. CRC Press Taylor & Francis Group Boca Raton London New York, 2009.
9. Wroblewska B., Kolakowski P., Pawlikowska K., Troszynska A., Kaliszewska A. (2009) Influence of the addition of transglutaminase on the immunoreactivity of milk proteins and sensory quality of kefir. Food Hydrocolloids 8 (23): 2434-2445.
10. Mandalari G., Adel-Patient K., Barkholt V., Baro C., Bennett L., Bublin M., Gaier S., Graser G., Ladics G.S., Mierzejewska D., Vassilopoulou E., Vissers Y. M., Zuidmeer L., Rigby N.M., Salt L.J., Defernez M., Mulholland F., Mackie A.R., Wickham M.S., Mills E.N. (2009) In vitro digestibility of beta-casein and beta-lactoglobulin under simulated human gastric and duodenal conditions: A multi-laboratory evaluation. Regul. Toxicol. Pharmacol. 55 (3): 372-381.

Collaboration partners

- Instituto de Agroquímica y Tecnología de Alimentos, (CSIC), Burjassot/ Valencia, Spain;
- Université du Québec à Montréal, Canada;
- Institute of Food Research, Norwich, United Kingdom.

Department of Chemistry and Biodynamics of Food

Division of Food Sciences
Tuwima 10 Str., 10-748 Olsztyn



Head
Henryk Zieliński, Prof.

Professors
Maria Soral-Śmietana, Prof.
Mariusz K. Piskula, Prof.

Assistant Professors
Wiesław Wiczowski, Ph.D.
Urszula Krupa-Kozak, Ph.D.
Małgorzata Wronkowska, Ph.D.
Anna Horszwald, Ph.D.

Assistant
Ewa Ciska, Ph.D.

Senior Specialists
Joanna Honke, Ph.D.
Dorota Szawara – Nowak, M.Sc.

Technologists
Natalia Bączek, M.Sc.
Joanna Topolska, M.Sc.

Ph.D. Students
Monika Jadacka, M.Sc.
Małgorzata Przygodzka, M.Sc.

Professor emeritus
Halina Kozłowska, Prof.

Assistant in the project
Damian Gorczyca, Ph.D.

Focus areas

Studies conducted in the Department of Chemistry and Biodynamics of Food are aimed at qualitative and quantitative changes in biologically-active compounds of plant origin taking place during technological processes. The Department is also focused on analysis of the bioavailability of these compounds in respect of changes proceeding in the food matrix upon technological processes. Investigations include determinations of phenolic compounds (phenolic acids, flavonoids, tannins) and sulphur compounds (glutathione, glucosinolates), inositol phosphates (IP-6 to IP-3), oligosaccharides (alfa galactosides), betalain pigments and antioxidative vitamins. Antioxidative properties of the above-mentioned compounds are studied *in vitro* and *ex vivo*, both following their isolation from the food product matrix, as well as in the presence of other substances naturally occurring in food stuff. Apart from changes occurring in natural bioactive compounds present in a food matrix, the formation of non-enzymatic reaction products is being analysed (early, advanced, final Maillard reaction products), especially in relation to antioxidative properties of technologically processed products. In terms of metabolomics studies, the research concerns mainly absorption and metabolism of phyto-compounds.

Furthermore, the Department works on developing modifications of technological processes aimed at maintaining the highest possible level of healthy phyto-compounds in products, such as enzymatic or controlled fermentations, germination of legume and cruciferous seeds, milling of cereals and bread baking. In addition, research undertaken by the the scientific of the Depart-



ment is focused on designing model studies associated with functional properties of bakery and pastry products enriched with polyphenols. What is more, the Department carries out studies on gluten-free products obtained on the basis of gluten-free formulas enriched with buckwheat flour. Gluten-free formulas enhanced by the calcium salts content, with increased bioavailability of calcium due to the supplementation of insulin, are investigated. The studies on baking products also include the possibility of using the nutritional components of milk from acid whey remaining after the production of curd cheese, concentrated by different membrane separation processes, as a functional supplement of wheat or wheat-rye bread.

Current research

1. Qualitative and quantitative analysis of bioactive compounds present in raw material and obtained product, especially in respect to their influence on antioxidative, anti-inflammatory, anticancer properties of bakery and pastry products, as well as processed cruciferous vegetables (including biologically active products of glucosinolates hydrolysis);
2. Influence of drying and thermal treatment on formation of Maillard reaction products in bakery and pastry products as well as in domestic plums and their dried form (prunes). The research is expanded to the analysis of volatile compounds and their influence on functional and sensory properties of model bakery and pastry products;
3. Analysis of model bakery products with reduced glycemic index and increased antioxidative potential. Studies regard following the influence of the digestion process under conditions simulating those of the human gastrointestinal tract on the starch profile in food stuff;
4. Study of food products and their components being able to inhibit protein glycation process - designing wheat-buckwheat products preventing 2-types diabetes;
5. A research project concerning coeliac disease that will evaluate the influence of gluten-free diet with optimal or deficient calcium supply and inulin addition on the expression of genes involved in the mechanism of active and passive calcium transport in the rat duodenum, profile of the caecum microflora of rats fed with the experimental diet, as well as the calcium absorption and transepithelial transport from caecum contents based



on *in vitro* experiment with Caco-2 cell culture;

6. Investigations ongoing in the area of metabolomics are focused on absorption and metabolism of phytochemicals, food compounds bioavailability, analysis of food intake biomarkers, impact of food consumption on consumer metabolome, permeation of phytochemicals across the blood-brain barrier and to the lungs.



Achievements

- determining the antioxidative potential of basic cereals (buckwheat > barley > oats > rye > wheat) and indicating that increased antioxidative potential of products obtained on their basis (breads) in relation to raw material (flour) used for baking is related to the formation of Maillard reaction products;
- development of indicators of antioxidative and reductive properties, as well as *in vitro* protein glycation inhibition as important criteria for assessing the functional properties of wheat-buckwheat breads being an example of polyphenol enriched food;
- investigation of dynamic changes of phytochemicals during germination of legume, cruciferous and buckwheat seeds, and optimization of this process aimed at maintaining the highest possible antioxidative properties of the sprouts;
- designing experimental gluten-free formulas enhanced with buckwheat flour and application of the nutritional components of milk from acid whey remaining after the produc-

tion of curd cheese as a functional component used for wheat bread and wheat-rye bread baking;

- analysis of selected flavonoids absorption process followed by a postulation of a sequence of their conjugation; development and implementation of procedures for non-clinical food phytochemicals bioavailability medical research with the participation of volunteers, as well as studies of phytochemicals bioavailability with animals.

Analytical facilities

1. HPLC gradient system with autosampler (LC-20, Shimadzu) coupled with DAD detector (SPD-M20A, Shimadzu) and mass spectrometer (QTRAP 5550 AB SCIEX, Shimadzu);
2. HPLC gradient system with autosampler (LPG-3400M, WPS-3000TSL, Dionex) coupled with multi-channel electrochemical coulometric detector (5600A, ESA) and fluorescence detector (FLD-3400RS, Dionex);
3. HPLC gradient system with autosampler (LC-10, Shimadzu) coupled with DAD detector (SPD-M10AVP, Shimadzu) and mass spectrometer (LCMS-QP8000α, Shimadzu);
4. HPLC gradient system with autosampler (LC-10, Shimadzu) coupled with UV-Vis detector (SPD-10A, Shimadzu) and fluorescent detector (RF-10AXL, Shimadzu);
5. HPLC gradient system with autosampler (LC-6, Shimadzu) coupled with UV-Vis detector (6AV, Shimadzu), fluorescent detector (RF-535, Shimadzu) and refraction index detector (RID-6A, Shimadzu);
6. HPLC isocratic system (LC-10, Shimadzu) coupled with two amperometric detectors (ICA-3000, TOA, Japan and ED40, Dionex);

**bioactive compounds,
Maillard reaction products,
antioxidative properties,
technological processes, functional
and rheological properties, enzymatic
hydrolysis *in vitro*, transportation of
calcium, bioavailability, metabolome**

Contact
Henryk Zielinski, Prof.
h.zielinski@pan.olsztyn.pl

7. Gas chromatograph equipped with MS and FID, ECD and FPD detectors; (7890A – Agilent Technologies, GC-14A – Shimadzu);
8. UV-VIS spectrophotometer with temperature-controlled measuring cell (UV-1601PC, Shimadzu), luminescent spectrophotometer (LS 50 B, Perkin Elmer), Photochem analyzer (Analytical Jena).

Selected papers

1. Zielinski H., Zielinska D., Kostyra H. (2012) Antioxidant capacity of a new crispy type food products determined by updated analytical strategies. *Food Chem.* 130: 1098-1104.
2. Skipor J., Misztal T., Piskula M., Wiczowski W., Thiery J.-C. (2012) Phytoestrogens and thyroid hormone levels in the cerebrospinal fluid of ewes fed red clover silage. *Small Ruminant Res.* 102: 157-162.
3. Piskula M.K., Murota K., Terao J. (2012) Bioavailability of Flavonols and Flavones, in "Flavonoids and Related Compounds: Bioavailability and Function" Series: Oxidative Stress and Disease, Eds. Spencer J.P.E., Crozier A., CRC Press, Taylor and Francis Group. 93-107.
4. Blaszcak W., Zielinska D., Zielinski H., Szawara-Nowak D., Fornal J. (2011) Antioxidant properties and rutin content of high pressure-treated raw and roasted buckwheat groats. *Food Bioprocess. Technol.* DOI: 10.1007/s11947-011-0669-5.
5. Krupa-Kozak U., Wronkowska M., Soral-Smietana M. (2011) Effect of buckwheat flour on microelements and proteins contents in gluten-free bread. *Czech J. Food Sci.* 29 (2): 103-108.
6. Wiczowski W. (2011) Garlic and Onion: Production, Biochemistry and Processing, In:

Handbook of Vegetables & Vegetable Processing, eds. Sinha N.K., Hui. Y.H., Özgül Evranuz E., Siddiq M., Ahmed J., Wiley-Blackwell, Ames, USA, 625-642.

7. Krupa-Kozak U., Troszynska A., Baczek N., Soral-Smietana M. (2011) Effect of organic calcium supplements on the technological characteristic and sensory properties of gluten-free bread. *Eur. Food Res. Technol.* 232 (3): 497-508, DOI: 10.1007/s00217-010-1421-5.
8. Zielinska D., Zielinski H. (2011) Antioxidant activity of flavone C-glucosides determined by updated analytical strategies. *Food Chem.* 124: 672-678.
9. Wiczowski W., Romaszko E., Piskula M.K. (2010) Bioavailability of Cyanidin Glycosides from Natural Chokeberry (*Aronia melanocarpa*) Juice with Dietary-Relevant Dose of Anthocyanins in Humans. *J. Agric. Food Chem.* 58: 12130-12136.
10. Martinez-Villaluenga C., Penas E., Ciska E., Piskula M.K., Kozłowska H., Vidal-Valverde C., Frias J. (2010) Time dependence of bioactive compounds and antioxidant capacity during germination of different cultivars of broccoli and radish seeds. *Food Chem.* 120 (3): 710-716.

Collaboration partners

- The Spanish National Research Council (CSIC): CIAL, ICTAN (Madrid) and IATA, Valencia, Spain;
- University of Helsinki, Institute of Biotechnology, Finland;
- Institute of Food Research, Norwich; King's College London and Nothumbria University, Newcastle, Great Britain;
- The University of Tokushima, Japan;
- Technion-Israel Institute of Technology, Department of Food Engineering and Biotechnology, Haifa, Israel;
- Product Design and Quality Management Group, Agrotechnology & Food Sciences Wageningen University, Agrotechnical Research Institute of Biotechnology (ATO-DLO), the Netherlands;
- HES-SO, University of Applied Sciences, Sion, Switzerland;
- The Unité de Nutrition Humaine, UNH, INRA, Centre de Recherché de Clermont-Ferrand, France;
- VUP Food Research Institute, Bratislava, Slovakia.

Department of Chemical and Physical Properties of Food

Division of Food Sciences
Tuwima 10 Str., 10-748 Olsztyn



Head
Ryszard Amarowicz, Prof.

Professor
Józef Fornal, Prof.

Associated Professor
Wioletta Błaszczak, Ph.D., D.Sc.

Assistant Professors
Magdalena Karamać, Ph.D., D.Sc.
Agnieszka Kosińska, Ph.D.
Tomasz Jeliński, Ph.D.

Senior Specialists
Jadwiga Sadowska, Ph.D.
Andrzej Ornowski, M.Sc.

Technologists
Kamila Penkacik, M.Sc.
Katarzyna Sulewska, M.Sc.

Ph.D. Students
Adrian Górecki, M.Sc.
Michał Janiak, M.Sc.

Assistant in the project
Valentina Kiseleva, Ph.D.

Focus areas

The research of chemical properties in the Department of Chemical and Physical Properties of Food include polyphenolic compounds of plant origin and their antioxidant properties analysed using several methods, such as ABTS, DPPH, FRAP, carotene bleaching and CL. What is more, antioxidant properties of protein hydrolysates are also investigated. HPLC and capillary electrophoresis methods are used for the analysis of bioactive compounds. The fluorescence and protein precipitation are applied to measure the interaction between phenolics and proteins. In terms of physicochemical properties of food, the research concerns analysis of the relation: microstructure – physical properties – food quality. Raw intermediate products and ready to use food stuffs of plant and animal origin are the main analytical materials used in the studies. What is more, the effect of variability of genetically and technologically induced physical properties of raw materials and food products are examined. Among the methods used, a special emphasis is put on the methods employed in the study of rheological properties. The results obtained from the rheological tests are confronted with the outcome of microstructure analysis conducted with the optical (LM) and scanning electron (SEM) microscopes. Microscopic pictures are transformed into the digits using digital image analysis (DIA), what in turn enables an objective and detailed description of the relationships undergoing investigation.

Current research

1. Obtaining starch carriers with a controlled release for biologically active compounds obtained under high hydrostatic pressure conditions;

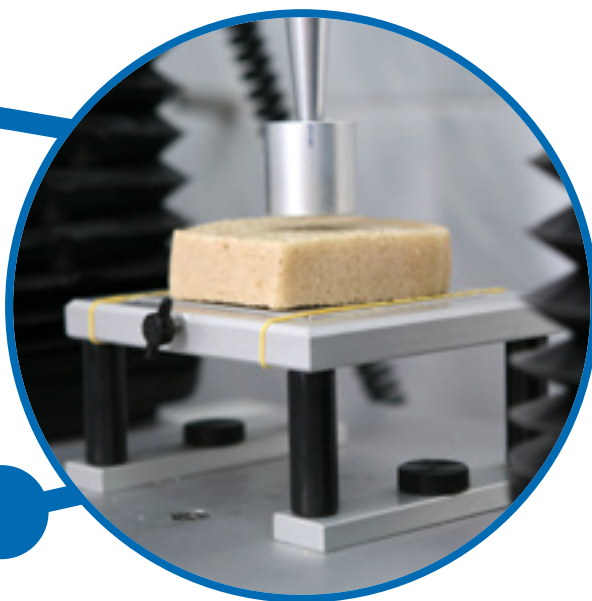


2. Development of novel products of instant-type using high hydrostatic pressure technology;
3. Application of high pressure to control the maturation and/ or extend the shelf life of selected beverages;
4. Determination of thermodynamic properties of raw materials, their changes under technological processes with emphasis put on the quality of the final product;
5. Development of quality standards for selected products of animal origin using digital image analysis (DIA);
6. Microscopic analysis of the raw material, its changes upon technological processes in relation to the texture properties of the final product;
7. Antioxidant activity and chelation properties of proanthocyanidins of green tea;
8. Antioxidant activity of flaxseed protein hydrolysates;
9. Antioxidant activity of flaxseed phenolic compounds in lipid systems;
10. Influence of bacteria on flaxseed phenolic compounds.

Achievements

- development of the algorithm for digital description of food structure indices (microstructure of wheat grain, porosity of bread, non-invasive detection of cereal grain insects, cheeses);
- calculation of the thickness of growth rings and semicrystalline lamellae clusters in starch granules using SEM, TEM and WAXS methods;
- discovery of the presence of caffeic acid

- in the flaxseed lignan macromolecule;
- application of a Sephadex LH-20 column chromatography for purification of flaxseed lignan (SDG);
- application of hydrolysis for enhanced antioxidant activity of flaxseed phenolic compounds.



Analytical facilities

1. Texture analyzer TA.HD Plus;
2. Scanning electron microscope JSM 5200 (JEOL Japan);
3. Optical microscope OLYMPUS BX 60;
4. Differential scanning calorimeter (DSC) Diamond (Perkin Elmer);
5. High hydrostatic pressure device U 33;
6. HPLC Shimadzi LC-10 system with DAD and RID;
7. Capillary electrophoresis Beckman P/ACE 5510 system with DAD;
8. Beckman DY 7500 UV-Vis spectrophotometer.

Selected papers

1. Blaszcak W., Bidzinska E., Dyrek K., Fornal J., Michalec M., Wenda E. (2011) Effect of phosphorylation and pretreatment with high hydrostatic pressure on radical processes in maize starches with different amylose contents, Carbohydrate Polymers 85: 86-96.
2. Kosinska A., Karamac M., Penkacik K., Urbalewicz A., Amarowicz R. (2011) Interactions between tannins and proteins isolated from broad bean seeds (Vicia faba Major) yield soluble and non-soluble

complexes. European Food Research and Technology 233: 213-222.

3. Amarowicz R. (2011) Lycopene as natural antioxidant. European Journal of Food Lipids and Technology 113: 675-677.
4. Stasiak M., Rusinek R., Molenda M., Fornal J., Blaszcak W. (2011) Effect of potato starch modification on mechanical parameters and granules morphology. Journal of Food Engineering 102: 154-162.
5. Blaszcak W., Bidzinska E., Dyrek K., Fornal J., Wenda E. (2010) EPR study of the influence of high hydrostatic pressure on the formation of radicals in phosphorylated potato starch. Carbohydrate Polymers 82: 1256-1263.
6. Amarowicz R., Estrella I., Hernández T., Robredo S., Troszynska A., Kosinska A., Pegg R.B. (2010) Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (Lens culinaris). Food Chemistry 121: 705-711.
7. Karamac M. (2010) Antioxidant activity of tannin fractions isolated from buckwheat seeds and groats. Journal of American Oil Chemists' Society 87: 559-566.
8. Craft B.D., Kosinska A., Amarowicz R., Pegg R.B. (2010) Antioxidant properties of extracts obtained from raw, dry-roasted, and oil-roasted US peanuts of commercial importance. Plant Foods for Human Nutrition 65: 311-318.
9. Markowski M., Bondaruk J., Blaszcak W. (2009) Rehydration behavior of vacuum-microwave-dried potato cubes. Dry Technology 27: 296-305.
10. Noda T., Isono N., Krivandin A.V., Shatalova O.V., Blaszcak W., Yuryev V.P. (2009) Origin

**food analysis,
microstructure, antioxidant
activity, microscopy,
chromatography, starch,
phenolic compounds**

Contact
Tomasz Jeliński, Ph.D.
t.jelinski@pan.olsztyn.pl



of defects in assembled supramolecular structures of sweet potato starches with

different amylopectin chain-length distribution. Carbohydrate Polymers 76: 400-409.

Collaboration partners

- Food Refrigeration and Computerized Food Technology Center, University College of Dublin, Ireland;
- Institute of Food Science, Technology and Nutrition, ICTAN-CSIC, Madrid; Institute of Agrochemistry and Food Technology, YATA, Valencia, Spain;
- Institute of Biochemical Physics, Moscow, Russia;
- Department of Food Science and Technology, University of Milano, Italy;
- Institute of Organic Chemistry and Centre of Phytochemistry, Sofia, Bulgaria;
- Department of Food Science and Technology, University of Georgia, Athens, USA;
- Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada.

Department of Biological Function of Food

Division of Food Sciences
Tuwima 10 Str., 10-748 Olsztyn



Head
Zenon Zduńczyk, Prof.

Professor
Jerzy Juśkiewicz, Prof.

Assistant Professors
Adam Jurgoński, Ph.D.
Monika Wróblewska, Ph.D.

Senior Specialist
Łucja Brzuzan, M.Sc.

Focus areas

The focus of research interests at the Department of Biological Function of Food is the effect of diet constituents on the functioning of the alimentary tract (i.e. absorptive functions of the small intestine and fermentation processes in the colon), usability of various nutritional interventions in health prophylaxis, and finally verification of their efficacy in reducing laboratory-induced metabolic disorders that commence pathogenic processes including a high level of free radicals, dyslipidemia and hyperglycemia. A special research area includes the assessment of alimentary tract functioning (including metabolic activity of colonic microflora) and the resultant changes in blood and tissue levels of those metabolites that may reduce or increase the risk of diet-related diseases. Experiments are conducted with conventional laboratory rodents, including rats with impaired metabolism (e.g. through induction of early or advanced diabetes) and livestock animals being research models of selected diseases or providing food products that play a significant role in human's diet. Investigations conducted at the Department are aimed at searching for new methods of the biological evaluation of functional components and special diets, with consideration given to the possibility of reducing diet-dependent metabolic disorders. What is more, the objective of experiments with livestock is to evaluate possibilities of improving the health quality of animal raw materials (meat and eggs).



Current research

1. Characteristics of metabolic effects of fermented seeds of soybean, flax and lupine and analysis of the usability of polyphenolic extracts from different fruits in the inhibition of postprandial hyperglycemia;
2. Investigations on the usability of polyphenols and dietary fibre from color fruits in stimulating the ecosystem of the gastrointestinal tract and minimizing effects of metabolic disorders using rats with induced inulin-resistance and early-phase diabetes;
3. Investigations on the physiological response of bodies of broiler chickens and laying hens to feeding with feed mixtures containing new-generation feed additives;
4. Characteristics of nutritional and health-promoting effect of bakery products enriched with whey in model experiments with rats;
5. Modeling the health quality of meat and eggs using phytobiotics, being effective sources of PUFAs and antioxidants, in poultry diets;
6. Characteristics of physiological effects of ellagitannin extracts from fruits of the rose family, dextrin preparations from potato starch, endogenous β -glucuronidases and β -glucosidases, and characteristics of nutritional and health-promoting properties of dairy products manufactured with the use of transglutaminase.

Achievements

- demonstrating that strawberry pomaces constitute a rich source of dietary fiber and bioactive polyphenolic compounds (ellagitannins and ellagic acids in particular) useful

in mitigation of metabolic disorders in rats fed a high-fructose diet. The strawberry pomaces have been reported to positively affect blood lipid profile, antioxidative status and insulinemia advancement;

- documenting significantly enhanced metabolism of quercetin and its glycosides in the blind gut of rat upon diet supplementation with prebiotic fructooligosaccharides;



- documenting the beneficial impact of mono- and dicaffeoylquinic acids and other polyphenols (chlorogenic and chicoric acid), obtained from roots, skins, leaves and seeds of chicory on the lipid profile and antioxidative status of the body as well as on the activity of bacterial β -glucuronidase in intestinal digesta and feces of rats;
- determining that the flavonoid fraction is primarily responsible for beneficial changes in blood lipid profile in rats fed a diet with apple pomace;
- showing that beetroot chips rich in betacyanins, betaxanthine and dietary fiber exert a positive effect on the blood lipid profile and antioxidative status of rat body.

Analytical facilities

1. Unicam Helios α spectroscope (determination of the activity of selected endogenous and microbiological enzymes in intestinal digesta);
2. Asys UVM340 plate reader;
3. Reflotron Plus (quantitative determination of biochemical blood markers using dry phase tests);

4. Odyssey – a laser system for detection and analysis of fluorescent objects in infrared;
5. Air-conditioned facilities of the Animal Laboratory with complete equipment for maintenance of a mother herd and for conducting studies with 120 individually-kept rats and 36 rats kept in metabolic cages.

Selected papers

1. Jurgonski A., Juskiewicz J., Zdunczyk Z., Krol B. (2012) Caffeoylquinic acid-rich extract from chicory seeds improves glycemia, atherogenic index, and antioxidant status in rats. *Nutrition* 28 (3): 300-306.
2. Juskiewicz J., Zary-Sikorska E., Zdunczyk Z., Krol B., Jaroslawska J., Jurgonski A. (2012) Effect of dietary supplementation with unprocessed and ethanol-extracted apple pomaces on caecal fermentation, antioxidant and blood biomarkers in rats. *Br. J. Nutr.* 107: 1138-1146.
3. Jurgonski A., Juskiewicz J., Kowalska K., Zdunczyk Z. (2012) Does dietary inulin affect biological activity of a grapefruit flavonoid-rich extract? *Nutr. Metab.* 9: 31.
4. Juskiewicz J., Zdunczyk Z., Bohdziewicz K., Baranowska M. (2012) Physiological effects of the dietary application of quark produced with enzyme transglutaminase as a sole protein source in growing rats. *Int. Dairy J.* 26: 155-161.
5. Wroblewska M., Brzuzan L., Zdunczyk Z. (2011) Effect of buckwheat sprouts and groats on the antioxidant potential of blood and caecal parameters in rats. *Int. J. Vit. Res.* 81** (5): 286-294.
6. Jaroslawska J., Juskiewicz J., Wroblewska M., Jurgonski A., Krol B., Zdunczyk Z.

**nutritional value,
nutrition physiology,
diet health quality, diet
supplements, metabolism, rats**

Contact
Zenon Zduńczyk, Prof.
zez@pan.olsztyn.pl



- (2011) Polyphenol-rich strawberry pomace reduces serum and liver lipids and alters gastrointestinal metabolite formation in fructose-fed rats, *J. Nutr.* 141 (10): 1777-1783.
7. Juskiewicz J., Milalla J., Jurgonski A., Krol B., Zdunczyk Z. (2011) Consumption of polyphenol concentrate with dietary fructooligosaccharides enhances cecal metabolism of quercetin glycosides in rats. *Nutrition* 27: 351-357.
8. Juskiewicz J., Zdunczyk Z., Zary-Sikorska E., Krol B., Milalla J., Jurgonski A. (2011) Effect of dietary polyphenolic fraction of chickory

- root, peel, seed and leaf extracts on caecal fermentation and blood parameters in rats fed diets containing prebiotic fructans. *Brit. J. Nutr.* 105: 710-720.
9. Jurgonski A., Milalla J., Juskiewicz J., Zdunczyk Z., Krol B. (2011) Composition of chicory root, peel, seed and leaf ethanol extracts and biological properties of their non-inulin fractions. *Food Techn. Biotechn.* 49 (1): 40-47.
10. Wroblewska M., Juskiewicz J., Wiczkowski W. (2011) Physiological properties of beetroot crisps applied in standard and dyslipidaemic diets of rats. *Lipids Health Dis.* 10: 178-185.

Collaboration partners

- Lithuanian Veterinary Academy in Kaunas, Lithuania;
- Department of Animal Science, University of Manitoba, Winnipeg, Canada.

Department of Biosensors

Division of Food Sciences
Tuwima 10 Str., 10-748 Olsztyn



Head
Jerzy Radecki, Prof.

Assistant Professor
Katarzyna Kurzątkowska, Ph.D.

Assistants
Kamila Malecka, M.Sc.
Urszula Jarocka, M.Sc.
Agata Jargiło, M.Sc.

Focus areas

The intensive development of science and technology has brought longevity and higher standards of living, but paradoxically has also generated new threats to human health. Good health is jeopardised by chemical compounds, both organic and inorganic, found in waste by-products of numerous technological processes. Moreover, pharmaceuticals and pesticides introduced into the food chain and their biological change by-products may have a negative impact on human life and the environment. Therefore, it is vital to formulate an integrated natural environment control system allowing continuous monitoring and facilitating observation of the movement of toxic and potentially toxic compounds in the environment, tracing how they are incorporated into the food chain and enabling their chemical analysis. To achieve this aim it is necessary to develop new analytical methods for chemical contamination control in food, water and the natural environment in which food is produced. The special attention is currently focused on the implementation of nanosystems and smart miniaturized systems in the food, natural environment and medical sectors. Joining this vivid area, we are working on the development of new materials/ analytical devices that could contribute to food and medical diagnosis applications. Tools will be designed to work as sensors of electrochemical modes of operation. Low cost, quick analysis and miniaturisation of analytical equipment justify such selection. The working mechanism of the proposed sensors is based on intermolecular (receptor – analyte) recognition processes occurring at organic /aqueous interfaces. The synthetic and naturally occurring receptors are applied



as the analytically active elements that decide about selectivity of analytical devices. In order to improve the analytical devices properties, such as sensitivity, durability and reusability, we are working on new materials for fabrication of matrix for proper receptors immobilization as well as for new efficient transducers.

Current research

1. Sensors and biosensors working based on redox active monolayers
 - gold electrodes modified with self-assembled monolayer created with porphyrine complexes with transition metals cations – destined for fabrication of genosensors;
 - gold electrodes modified with self – assembled monolayer created with ligands chating the transition metal cations destined for oriented immobilization of His-tagged proteins;
 - genosensors created based on ssDNA strand functionalized with redox active compounds destined for detection of viruses.
2. Sensors and biosensors working based on ion-channel mimetic mode
 - gold electrodes modified with self – assembled monolayer created with calixarenes destined for determination of neurotransmitters in human plasma;
 - gold electrodes modified with self – assembled monolayer incorporated juvenile hormone binding protein for screening of its interactions with hormones or their analogs;
 - immunosensors for detection of Prunus Necrotic Ringspot viruses in plant extracts;
 - immunosensors for detection of Avian Influenza virus H5N1.

Achievements

- Development of electrochemical sensor for determination of dopamine in human plasma.
A thio-derivative of corrole has been immobilized on the surface of gold electrodes via covalent Au-S bonds acting as a selective receptor for dopamine. The supramolecular complex formation on the electrode surface between the corrole host and dopamine guest via hydrogen bonding was detected by Osteryoung square wave voltammetry and by electrochemical impedance spectroscopy using $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ as the redox marker. The proposed sensor, displaying good sensitivity towards DA (detection limit in 10^{-12} M range), good selectivity (human plasma components have no influence on dopamine deter-



mination), and simple and quick procedure of electrode preparation, could be applied for the determination of dopamine in clinical analysis.

- Development of immunosensors for determination of Plum Pox Virus in plant extracts.
The immunosensor was based on gold electrodes modified with: 1,6-hexanedithiol, gold nanoparticles, anti-PPV IgG polyclonal antibody and BSA. It was used for determination of the virus in the extracts from plum (*Prunus domestica*) and tobacco (*Nicotiana benthamiana*) leaves. The immunosensor displayed very good detection limit 10 pg/ml and wide dynamic range from 10 pg PPV/ ml to 200 pg/ml. The presence of extract from plant materials has no influence on the immunosensor response. The immunosensor was capable of discriminating between samples from healthy plants and samples containing 0.01% of extract from infected plant material.
- Development of genosensors for determination of Avian Influenza Virus H5N1.
The genosensor, fabricated basing on self-assembling thiolated ssDNA probe on the gold electrode surface, displayed good sensitivity, namely detection limits of 2.2×10^{-11} M and of 2.4×10^{-11} M for the complementary 20-mer ssDNA and for the double stranded 181-bp DNA containing 20 nucleotides complementary to the target at 3'-end, respectively. The genosensor also shows good selectivity. Non-complementary target DNA generated weak responses. One of the main



advantages of the genosensor is the suitability for determination of PCR products (double stranded DNA fragments) and discrimination of different positions of the complementary parts. Considering the above analytically important parameters, the genosensor presented could be successfully applied for detection of the H5N1 virus.

- Development of novel voltammetric biosensor for determining acrylamide in potato chips extracts.

A carbon-paste electrode modified with hemoglobin can be used for direct electrochemical determination of acrylamide. The base of the proposed biosensor is the formation of a hemoglobin-acrylamide adduct, which affects the redox properties of the heme part of hemoglobin. The decrease in the peak current of the reduction process ($\text{Hb-Fe}^{3+}/\text{Hb-Fe}^{2+}$) occurring upon increasing concentration of acrylamide could be treated as an analytical signal.

The biosensor proposed was effective regarding the following parameters: very good sensitivity towards acrylamide (detection limit is 1.2×10^{-10} M), very good selectivity (the matrix, water extract from potato crisps – has no influence on the electrochemical signal generated in the presence of acrylamide in solution), very simple procedure of sample preparation.

Therefore, the carbon-paste electrodes modified with hemoglobin might be recommended for direct electrochemical determination of acrylamide in the food samples.

- Development of potentiometric sensors for determination of undissociated forms of phenol derivatives and diaminobenzene isomers.

The main achievement is proving that membranes modified with hosts possessing phenolic groups generate cationic potentiometric responses upon stimulation by uncharged guests possessing NH_2 groups. The base of the potentiometric signal generation relies on the proton transfer from the aqueous phase on the supramolecular complexes located at the interface.

It can be concluded that the generation of potentiometric signals by uncharged molecules is governed by the transport of protons between the aqueous and membrane/aqueous interface. The acidity/basicity of the hosts decide about the direction of the transfer of protons.

Analytical facilities

1. Electrochemical measurement system AUTO-LAB, Eco Chemie;
2. Labs, Inc. EMF16 Precision Electrochemistry EMF Interface; Lawson;
3. UV-VIS spectrophotometer SHIMADZU;
4. Quartz Crystal Microbalance (QCM) 410 CH Instruments ;
5. OCA 15 - for wettability angels measurements DataPhysic;
6. Atomic force microscope (AMF) Qusant;
7. Scanning tunnelling microscope (STM) Quesant;
8. Electrochemical Scanning Microscope, SCV 370, Princeton Applied Research;
9. Surface plasmon resonace (SPR), AUTO-LAB, Eco Chemie.

**sensors, biosensors,
redox active monolayers,
food analysis, protein-
protein, proteins - small molecules
interactions , DNA probes**

Contact
Jerzy Radecki, Prof.
j.radecki@pan.olsztyn.pl

Selected papers

1. Ostatná V., Černocká H., Kurzatkowska K., Paleček E. (2012) Native and denatured forms of proteins can be discriminated at edge plane carbon electrodes. *Analytica Chimica Acta* 735: 31 – 36.
2. Malecka K., Grabowska I., Radecki J., Stachyra A., Gora-Spachacka A., Sirko A., Radecka H. (2012) Voltametric Detection of a Specific DNA Sequence of Avian Influenza Virus H5N1 Using HS-ssDNA Probe Deposited onto Gold Electrode. *Electroanalysis* 24: 439 – 446.
3. Jarocka U., Wasowicz M., Radecka H., Malinowski T., Michalczuk L., Radecki J. (2011) Impedimetric Immunosensor for Detection of Plum Pox Virus in Plant Extract. *Electroanalysis* 23: 2197 – 2204.
4. Nulens W., Grabowska I., Ngo H.T., Dehaen W., Radecka H., Radecki J. (2011) Determination of the surface acidity of a free-base corrole in a self-assembled monolayer. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 71: 499 – 505.
5. Poduval R., Kurzatkowska K., Stobiecka M., Dehaen W.F.A., Dehaen W., Radecka H., Radecki J. (2010) Systematic study of interaction of the neutral form of anilines with undecylcalix[4]resorcinarene derivatives by means of potentiometry. *Special issue of Supramolecular Chemistry* 22: 412 – 418.
6. Ito T., Grabowska I., Ibrahim S. (2010) Chemical force-microscopy for materials characterization. *Trends in Analytical Chemistry* 29: 225 – 233.
7. Kurzatkowska K., Dolusic E., Dehaen W.,

Sieron - Stoltny K., Sieron A., Radecka H. (2009) Gold Electrode Incorporating Corrole as an Ion-Channel Mimetic Sensor for Determination of Dopamine. *Analytical Chemistry* 81: 7397 – 7405.

8. Viswanathan S., Radecka H., Radecki J. (2009) Electrochemical biosensors for food analysis. *Chemical Monthly* 140: 891 – 899.
9. Viswanathan S., Voloshin Y.Z., Radecka H., Radecki J. (2009) Single molecular switch based on thiol tethered iron(II) clathrochelate on gold. *Electrochimica Acta* 54: 5431 – 5438.
10. Viswanathan S., Radecka H., Radecki J. (2009) Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited vertically assembled carbon nanotubes wrapped with ssDNA. *Biosensors and Bioelectronics* 29: 2772 – 2777.



Collaboration partners

- School of Chemistry, Southampton University, Great Britain;
- Department of Chemistry, The University of Leuven, Belgium;
- Department of Chemistry, School of Science, The University of Tokyo; Research Institute of Pharmaceutical Sciences, Musashino University; Measurement Technology Group, Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan;
- University of Duisburg-Essen, Germany;
- Institute of Biophysics of Academy of Sciences of Czech Republic, Brno; Prague Institute of Chemical Technology, Czech Republic;
- Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia;
- Department of Chemistry of the State University of New York, Potsdam, USA.

Integrated Laboratory of Bioelectroanalysis

Division of Food Sciences
Tuwima 10 Str., 10-748 Olsztyn



Head
Hanna Radecka, Prof.

Assistant Professor
Iwona Grabowska, Ph.D.

Assistants
Edyta Mikuła, M.Sc.
Magdalena Zborowska, M.Sc.

Ph.D. Student
Justyn Wojtasik, M.Sc.

Focus areas

Alzheimer's disease results in a progressive loss of cognitive function and dementia affecting 36 million people, with the prevalence expected to double every 20 years. Therefore, the development of methods for early diagnosis, as well as for prevention is very important.



We have joined to this vivid research area, working on the development of the new analytical tools suitable:

- for determination of possible biomarkers of Alzheimer's and other neurodegenerative diseases present in human plasma (possible application in early diagnosis);
- for investigation of interactions between proteins and small organic molecules – potential



drugs (possible application in the prevention of neurodegenerative diseases).

Very promising research direction in this field is connected with electrochemical biosensors based on bi-functional redox active layer immobilized on the electrode surface. Such layers could serve as a transducer as well as an analytically active element. The redox centres, located inside the active sensing layers, are responsible for transduction of signals coming from recognition process to the analytical readable signals.

The features of redox active SAMs are mostly influenced by the distance between the redox centre and electrode, structure of bridge connecting the centre and electrode, as well as the molecular environment around the redox centre.

The crucial parameter deciding about the sensitivity and selectivity of biosensors based on re-

dox active layer containing the proteins applied as the recognition elements is their oriented immobilization on the electrode surface.

The research team of the Laboratory is working on new systems suitable for oriented immobilization of his-tagged proteins, based on thiol derivative of dipyrromethene-Cu(II) complex deposited on gold electrode surface and other related compounds.

Current research

1. Study of the interactions between V domain of Receptor for Advanced Glycation End Products (RAGE) and A β oligomers in the presence of selected alkaloids.

The receptor for advanced glycation end-products (RAGE) has been shown to interact with A-beta and to modulate A-beta trans-



port across the blood-brain barrier. Some evidences, recently published, suggest that RAGE is a key player in the signalling pathways triggered by A β and S100 proteins in AD. The objective of the research conducted is to establish the relationship between the structure of selected alkaloids and their influence on the kinetics of interactions between RAGE and A- β peptide.

This research will be performed with electrochemical techniques, surface plasmon resonance, and atomic force microscopy.

2. Study of the interactions between Janus kinase (JAKs) and potential inhibitors.

The kinases play the critical roles in fundamental cellular processes, such as cell cycle, cell division, differentiation and apoptosis. Searching of their efficient inhibitors - potential anticancer drugs - is very an intensively developing research field. The Laboratory is working on the fabrication of analytical tools suitable for screening of the interactions between his-tagged kinases and their potential inhibitors. The redox active monolayer was deposited onto gold electrode by progressive modification of thiol derivative of compounds chelating transition metal cations that may be applied for oriented immobilization of his-tagged kinase protein. The biosensors obtained are used for study of interactions between his-tagged kinase protein immobilized on the redox active surface and its inhibitors present in the aqueous solution. Such system mimics physiological conditions well and may be, therefore, used in the development of new drugs.

Achievements

- development analytical assay for determination of Association constants of alkaloids to amyloid β peptide determined by electrochemical impedance spectroscopy;
- development of analytical tool based on Surface Plasmon Resonance for screening the influence of naturally occurring compounds on the aggregation of amyloid- β peptide (A β 40);
- stable and oriented immobilization of his-tagged Rio1 protein on the surface of redox active monolayer incorporated with iminodiacetic acid -Cu(II) complex.

Analytical facilities

1. Electrochemical measurement system AUTO-LAB, Eco Chemie;
2. Potentiostat (10 -electrodes) - Donaulab.

Selected papers

1. Malecka K., Grabowska I., Radecki J., Stachyra A., Gora-Sochacka A., Sirko A., Radecka H. (2012) Voltametric Detection of a Specific DNA Sequence of Avian Influenza Virus H5N1 Using HS-ssDNA Probe Deposited onto Gold Electrode. *Electroanalysis* 24: 439 - 446. IF 2.87.
2. Krazinski B. E., Radecka H., Radecki J. (2011) Surface Plasmon Resonance Based Biosensors for Exploring the Influence of Alkaloids on Aggregation of Amyloid- β Peptide. *Sensors* 11: 4030 - 4042. IF 2.94.
3. Grabowska I., Radecka H., Burza A., Radecki J., Kaliszan M., Kaliszan R. (2010) Association constants of pyridine and piperidine alkaloids to amyloid beta-peptide determined by electrochemical impedance



spectroscopy. *Current Alzheimer Research* 7: 165 - 172. IF 4.98.

4. Szymanska I., Radecka H., Radecki J., Kaliszan R. (2007) Electrochemical Impedance Spectroscopy for Study of Amyloid β -Peptide

Interactions with (-) Nicotine Ditartrate and (-) Cotinine. *Biosensors and Bioelectronics* 22: 1955 - 1960. IF 5.42.

analytical tools, neuro-degenerative diseases, early diagnosis, prevention

Contact
Hanna Radecka, Prof.
h.radecka@pan.olsztyn.pl

Collaboration partners

- Department of Chemistry, The University of Leuven, Belgium;
- Institute of Biophysics of Academy of Sciences of Czech Republic, Brno; Prague Institute of Chemical Technology, Czech Republic;
- School of Chemistry, Southampton University; Great Britain;
- Department of Chemistry, University of Duisburg-Essen; Germany;
- Department of Chemistry, School of Science, The University of Tokyo; Research Institute of Pharmaceutical Sciences, Musashino University, Measurement Technology Group, Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan;
- Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia;
- Department of Chemistry of the State University of New York, Potsdam, USA.

Department of Prophylaxis of Metabolic Diseases

Division of Food Sciences
24a M.C. Skłodowskiej Str., 15-276 Białystok



Head
Marek Strączkowski, Prof.

Assistant Professor
Agnieszka Nikołajuk, DM, Ph.D.

Assistants
Remigiusz Filarski, M.Sc.
Radosław Majewski, M.Sc.

Assistant Professor in the project
Monika Karczewska-Kupczewska, DM, Ph.D.

Focus areas

The principal aims of the scientific research conducted in the Department of Prophylaxis of Metabolic Diseases are: 1) identification of early markers of insulin resistance, a major pathogenetic factor of type 2 diabetes and other diseases 2) finding new pathogenetic pathways leading to an impaired insulin action, what may contribute to establishing novel methods for treatment of type 2 diabetes, metabolic syndrome and associated disorders. Within the framework of the research being carried out, individuals at risk of developing type 2 diabetes, like subjects with positive family history of the disease or overweight/obese subjects with normal or impaired glucose tolerance, undergo examination. These groups are compared with lean healthy controls. The scientific team of the Department measure insulin sensitivity with the euglycemic hyperinsulinemic clamp technique and use indirect calorimetry to measure glucose and lipid oxidation. Furthermore, biopsies of subcutaneous adipose tissue and vastus lateralis muscle are performed, and peripheral blood mononuclear cells (PBMC) are isolated from the blood. In the tissues, mRNA is isolated and the expression of the selected genes is measured with the Real Time PCR. These techniques allow for the study of molecular mechanisms of insulin resistance. The Department is also conducting a weight loss program for obese subjects. Every two weeks each participant is given a detailed and personalized dietary advice. Additionally, a subgroup of participants, chosen randomly, takes a dietary supplement. At the beginning of the trial and after 12 weeks, body mass, body composition, insulin sensitivity, substrate oxida-



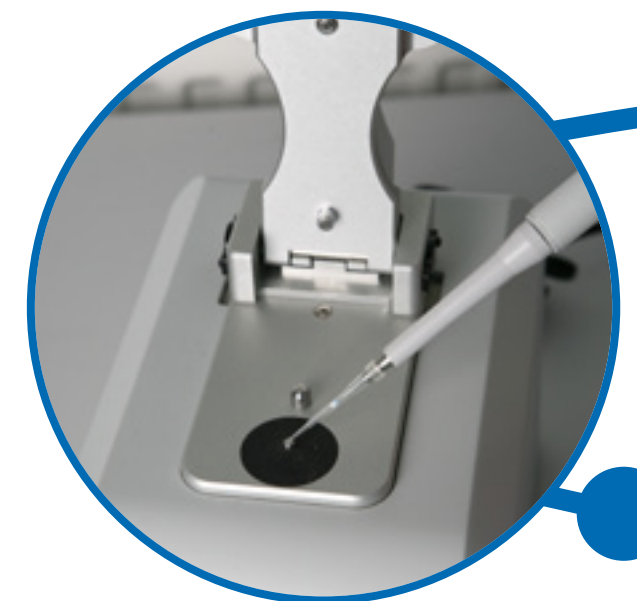
tion and the gene expression in adipose tissue and the PBMC are measured.

Current research

1. Regulation of glycoprotein 130 signalling pathway in cultures of skeletal muscle cells obtained from subjects with various degrees of insulin sensitivity;
2. Evaluation of the influence of beta-glucan 1.3D-1.6D, added to the low-calorie diet, on the amount of visceral adipose tissue, insulin sensitivity and expression of particular proinflammatory cytokines in adipose tissue and peripheral blood mononuclear cells in obese subjects with normal glucose tolerance.

Achievements

- demonstrating that the increased serum





free fatty acids concentration decreases circulating brain-derived neurotrophic factor. This might indicate a link between insulin resistance and neurodegenerative disorders.

Analytical facilities

1. NanoDrop 2000;
2. Centrifuge Eppendorf 5430R;
3. Thermo Stat Plus;
4. Body Composition Analyzer BioScan 920-2 Maltron;
5. AutoFlow NU-4950 Oxygen and Humidity Control Water Jacket CO2 Incubator;
6. CellGard ES NU-480 Class II, Type A2 Biosafety Cabinet;
7. Shaking Water Bath BS-11;
8. Centrifuge MiniSpin Plus (with standard rotor);
9. Mini – PROTEAN Tetra Cell vertical gel electrophoresis with accessories.

***insulin sensitivity, obesity,
type 2 diabetes, adipose
tissue, skeletal muscle,
weight loss***

Contact
Marek Strączkowski, Prof.
m.strackowski
@pan.olsztyn.pl



Selected papers

1. Piskula M.K., Strączkowski M., Żmudzki J., Osek J., Niemczuk K., Horbańczuk J.O., Skomiał J. (2011) Charakterystyka czynników decydujących o bezpieczeństwie konsumentów i jakości prozdrowotnej żywności. Pol J Agronomy 7: 82-91.
2. Karczewska-Kupczewska M., Kowalska I., Nikolajuk A., Adamska A., Zielinska M., Kaminska N., Otziomek E., Gorska M., Strączkowski M. (2012) Circulating brain-

derived neurotrophic factor concentration is down regulated by Intralipid/heparin infusion or high-fat meal in young healthy male subjects. Diabetes Care 35: 358-362.

3. Karczewska-Kupczewska M., Lelental N., Adamska A., Nikolajuk A., Kowalska I., Gorska M., Zimmermann R., Kornhuber J., Strączkowski M., Lewczuk P. (2012) The influence of insulin infusion on the metabolism of amyloid β peptides in plasma. Alzheimer's and Dementia.

Collaboration partners

- Universities of Girona, Tarragona and Barcelona, Spain;
- University of Erlangen-Nuremberg, Germany.

Laboratories

In Vitro and Cell Biotechnology Laboratory

Division of Reproductive Biology
7 Bydgoska Str., 10-243 Olsztyn



Head
Gabriel Bodek, Ph.D.

Specialist
Joanna Bukowska, M.Sc.

The studies carried out in the In Vitro and Cell Biotechnology Laboratory (core technology facility) focuses on *in vitro* cell cultures, flow cytometry and inverted confocal microscopy. The task of the Laboratory is to support the research conducted at the Institute, and to lead cooperation with similar scientific units. Using advanced methods of molecular biology and *in vitro* techniques, the Laboratory specializes in establishing of immortal cell lines. After the initial genotype characterization, the cells are subjected to further analysis or selection based on individual characteristics. The main objectives of the Laboratory include isolating and fully characterizing stem cells derived from the reproductive tract of farm animals, as well as studying the processes of carcinogenesis and developing new research models based on *in vitro* co-cultures. The research conducted and their orientation will allow for the implementation of the so-called good practice within the area of cooperation between science and economy.

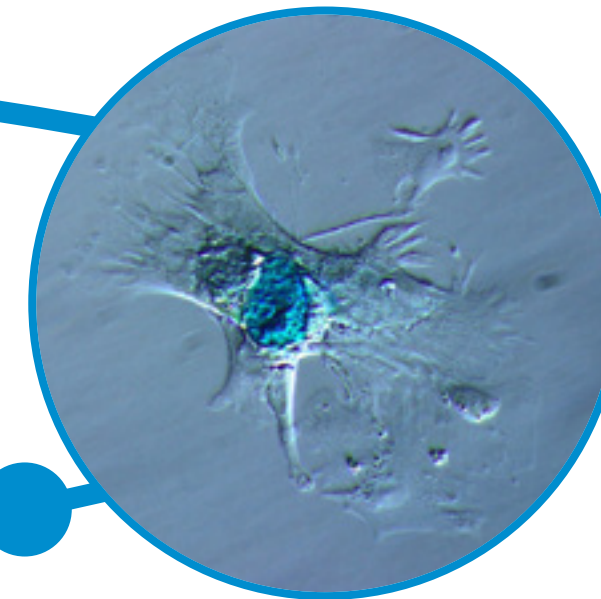
Research offer

The laboratory offers the following services:

1. Flow cytometry
 - estimation of cells proliferation (cell growth cycle analysis of different cells from *in vitro* cultures);
 - analysis of cell apoptosis (anexin V);
 - sorting of labeled cells or molecules.
2. Confocal microscopy
 - high resolution imaging of objects in 3D (optional with time-lapse imaging).
3. Microdissection – MMI system
 - contamination-free isolation of selected biological material;
 - isolation of single cells or tissue fragments from fixed specimens;
 - isolation of cells or cell clones from intravital cultures.
4. Cells cultures
 - cell clones isolation;
 - cell lines immortalization;
 - lipotransfection;
 - electroporation;
 - vector transfection;
 - preservation in liquid nitrogen;

Analytical facilities

- FACS Aria II cell sorter;
- Inverted confocal microscope Nikon Eclipse Ti-E;
- Laser microdissection – MMI system;
- Laminar flow chamber;
- CO2/O2 incubators.



**stem cells, flow cytometry,
pig endometrium**

Contact
Gabriel Bodek, Ph.D.
g.bodek@pan.olsztyn.pl



Molecular Biology Laboratory

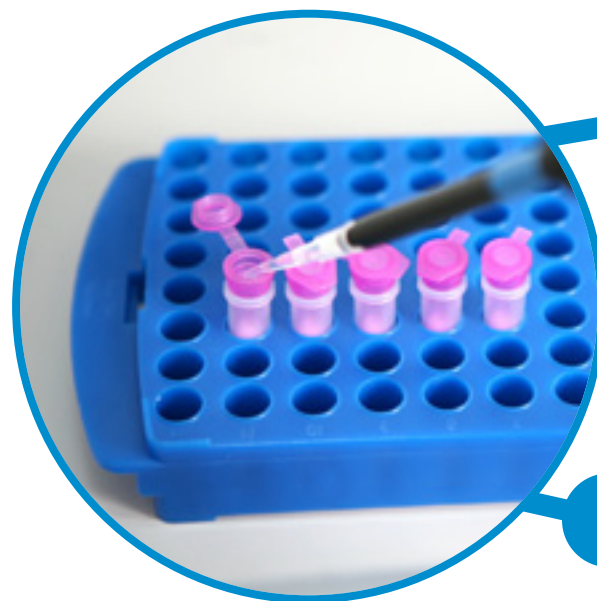
Division of Reproductive Biology
7 Bydgoska Str., 10-243 Olsztyn



Head
Monika Kaczmarek, Ph.D., D.Sc.

Senior Technician
Marta Romaniewicz, M.Sc.

Laboratory of Molecular Biology (LMB) was inaugurated within a project entitled Modernization of integrated educational and research laboratories in the Centre of Excellence BIOANIREP. LMB is dedicated to basic and applied molecular biology research, as well as service provision in terms of laboratory analyses using different molecular biology techniques. The overarching aim of the LMB is to popularize molecular biology techniques and to implement new innovative molecular biology methods and analyses into research carried out in the Centre of Excellence BIOANIREP, national and international co-operation networks and in the individual departments of the Institute. The LMB provides an unsurpassed environment for both young and established researchers, who wish to introduce molecular biology techniques to their research activity. Additionally, the Laboratory is as an educational facility, offering courses or consultations concerning molecular biology and provides advice and assistance in planning new experimental approaches based on up-to-date molecular techniques.

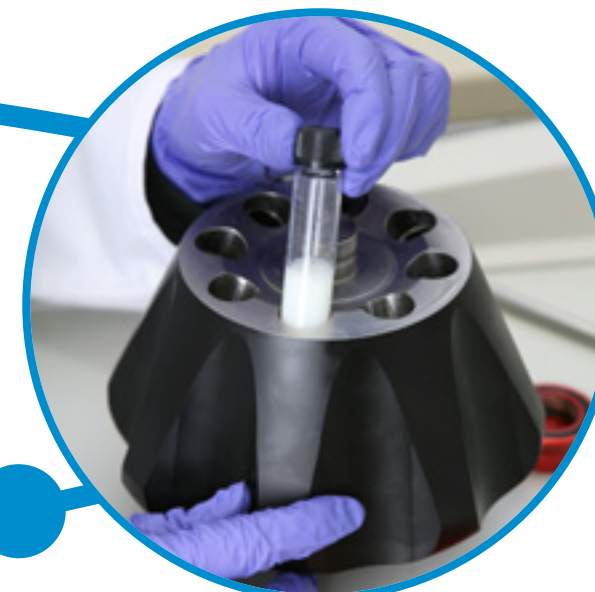


6. Global analysis of gene expression with data mining.

Basing on a considerable scientific experience the Laboratory team offers a training on the application of molecular biology techniques, such as real-time PCR (qPCR), in research and/or diagnostic approaches. Courses are divided into two parts: 1) lectures broadening knowledge about molecular biology techniques that can be implemented into everyday research and/or diagnostic practices, and 2) practical exercises e.g. qPCR assays on Applied Biosystems Sequence Detection systems (HT7900, HT7300, Viia 7).

Analytical facilities

- Real-time PCR Sequence Detection Systems (ABI HT7300, ABI HT7900, ABI Viia 7);
- SensoQuest hightech gradient thermocyclers;
- BIO-RAD VersaDoc MP4000 Imaging System;
- Agilent Technologies 2100 Bioanalyzer;
- Thermo Scientific Spectrophotometer NanoDrop;
- MP Biomedicals Homogenizer Fast Prep®-24;
- Genevac miVac Sample Concentrator;
- Beckman Optima L-100 XP Ultracentrifuge system;



Research and training offer

Laboratory services:

1. SDS-PAGE and Western blot;
2. sRNA, RNA, DNA isolation;
3. RNA, sRNA, DNA quantity, quality and purity assessment;
4. Nucleic acids electrophoresis;
5. Real-time PCR;

- Agilent Technologies High-Resolution Microarray Scanner;
- Thermo Scientific KingFisher®ML and QIA-GEN QIAcube automatic nucleic acids and protein Isolation Stations;
- Agilent Technologies Automated Liquid Handling Platform BRAVO.

**molecular biology,
reproductive biology, gene
expression, microRNA, early
pregnancy, embryo, uterus**

Contact
Monika M. Kaczmarek,
Ph.D., D.Sc.
lbm@pan.olsztyn.pl



Microbiological Laboratory

Division of Food Sciences
10 Tuwima Str., 10-748 Olsztyn

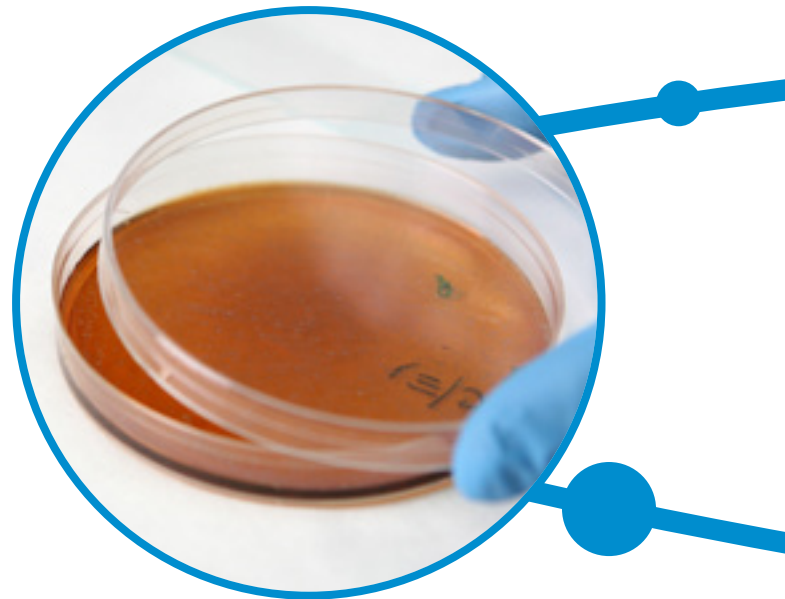


Head
Anna Majkowska, Ph.D.

Technologists
Danuta Rostek
Zdzisław Walo

Senior Technician
Ewa Łoś

The Microbiological Laboratory supervises 1000 bacteria strains collection. The majority of collection (c.a. 400 strains) constitutes probiotic Bifidobacterium and Lactobacillus strains isolated from selected media.



Research offer

The main research activities of the laboratory include:

1. Storing the strains with good survival rate and desirable properties;
2. Making the strains available to the all interested parties;
3. Searching for new strains with useful properties, their subsequent classification and identification, as well as selecting the proper storage conditions in the laboratory.

What is more, the Laboratory staff participates also in the studies carried out in other departments of the Institute. The research carried out is related to the main scientific areas of the Division of Food Sciences and concentrate on:

1. Determination of qualitative and quantitative changes of endogenous intestinal microbiota;
2. Interactions of gut microecosystem with antioxidants present in food;
3. Application of Lactobacillus and Bifidobacterium strains to decrease the immunoreactive properties of milk proteins.



In addition, the Laboratory carries out analyses ordered by the outer clients. It offers the microbiological analyses of foods and feeds in respect to the individual requirements of the clients. Furthermore, the Laboratory staff offers assistance in the selection of proper experimental methods and interpretation of the results.

Analytical facilities

- Analytical facilities;
- BIOSTAT C (Brown Biotech International) – bioreactor for bacterial biomass multiplying with sterilization and gas-mix systems;
- Tangential Filtration System “ProFlux” (Amicon) for bacterial biomass concentration;
- Freeze-drier FD 8-55 for the bacterial strains preservation;
- Computer equipment for microscopy analysis (Nikon).

*strains of bacteria,
microbiota, probiotics*

Contact
Anna Majkowska, Ph.D.
a.majkowska@pan.olsztyn.pl



Sensory Laboratory

Division of Food Sciences
10 Tuwima Str., 10-748 Olsztyn



Head
Agnieszka Troszyńska, Ph.D., D.Sc.

Senior Specialists
Beata Szmatowicz, Ph.D.
Grzegorz Lamparski, Ph.D.
Anita Ostaszyk, M.Sc.

Technologist
Julia Majewska, M.Sc.

Activities undertaken in the Sensory Laboratory are concerned with the research on the sensory quality of conventional, health-promoting and special-purpose food and its changes under different quality of raw materials, varied parameters of production, conditions and time of storage, and other factors. A part of the research performed is closely connected with scientific directions followed in other Departments of the Division of Food Sciences of the Institute. The Laboratory is also in a close cooperation with national and international research institutions and industrial companies. Sensory analyses are performed by a panel of experts trained and monitored according to international standards (ISO 8586-2:1996). The Research conducted are focused on:

1. Determining relationship between sensory quality and the content of various groups of bioactive compounds (phenolic acids, flavonoles, tannins), which affect negative sensory attributes such as bitterness and astringency;
2. Finding ways of masking negative sensory characteristics in order to fully benefit from polyphenolic antioxidants contained in food;
3. Developing and improving methods of sensory analyses using multivariate statistical techniques.

Training and research offer

Sensory Laboratory performs sensory analyses of food and organizes theoretical and practical sensory training. These include:

- selection, training and monitoring of the evaluation team;
- differential methods with a statistical interpretation of the results;
- quantitative methods with a statistical interpretation of the results;
- methods of a descriptive sensory analysis with a statistical interpretation of the results.

Apart from research tasks, the Laboratory offers a wide range of educational and service activities for the industry. These entail lectures, trainings, consultations and expertise in the area of sensory analysis, as well as assistance in the organization and management of innovative sensory laboratories.

The multivariate statistical techniques used in the interpretation of research results include:

1. Variation analysis (single and multiple factor analysis);
2. Regression analysis (simple and multiple);
3. Principal Component Analysis – PCA;
4. Cluster analysis;
5. Partial Least Square Regression – PLS;
6. Preparation of a preference map on the basis of analytical sensory results and consumer evaluations- preference mapping.

The use of these techniques has a considerable practical significance as they combine 2 different types of evaluation methods – consumer preference data and descriptive sensory information, helping to establish which sensory characteristics determine acceptance of the product and to identify consumer groups with similar preferences.

Analytical facilities

Sensory Laboratory with 12 individual boxes and a professional sensory panel fulfils requirements of the international standards (ISO 8589:1998). It is equipped with a computerized system for planning and realization of sensory evaluations (FIZZ, Biosystemes, France) with use of various methods and software to enable a statistical analysis and a graphic representation of the research obtained.



**sensory analysis,
consumer tests,
sensory laboratory, FIZZ,
multidimensional statistical
analysis, preference mapping**

Contact
Agnieszka Troszyńska,
Ph.D., D.Sc.
a.troszynska@pan.olsztyn.pl



Animal Laboratory

Division of Food Sciences
10 Tuwima Str., 10-748 Olsztyn



Head
Przemysław Zduńczyk, M.Sc.

Senior specialist
Irena Godycka-Kłós, M.Sc.



CV type conventional Animal Lab is located in the building of Division of Food Sciences. The first lab with an area of 120 m² was established in 1998 thanks to the financial support of the Foundation for Polish Science. In 2011 Laboratory's area was increased to 150 m² and thoroughly modernized with the grant from the Ministry of Science and Higher Education, fulfilling all the requirements for modern facilities of this type.

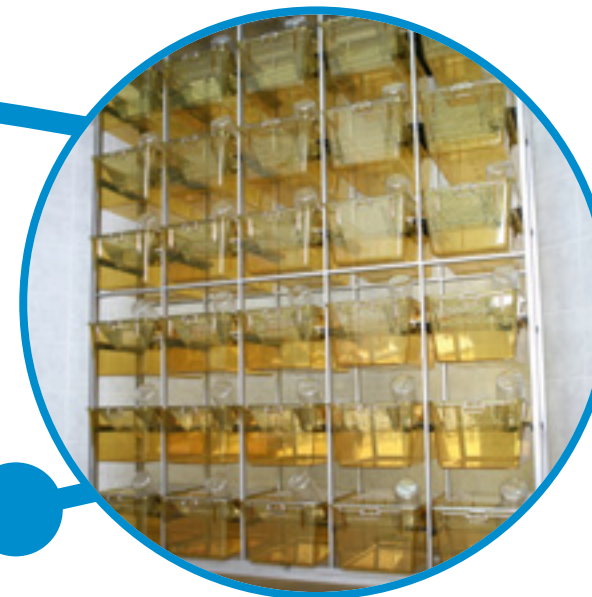
Research offer

1. Possibility of growing rats for experiments;
2. Taking care of the animals during the testing period;
3. Conducting nutrition experiments according to the agreed terms.

Analytical facilities

Animal Laboratory is divided into:

- 2 air-conditioned rooms to keep the mother herd of Wistar rats;
- 2 air-conditioned rooms to conduct studies with 130 individually-kept rats and 36 rats kept in metabolic cages;
- 1 air-conditioned room for studies on mice, with 60 cages for individual or group experiments, and 64 individually ventilated cages;
- 2 individually air-conditioned rooms for studies on mice, with 210 cages for individual or group experiments;
- surgery room;
- room for diet preparation;
- cage washing room;



- storage room;
- utility room.

Facilities of Animal Lab are currently used by:

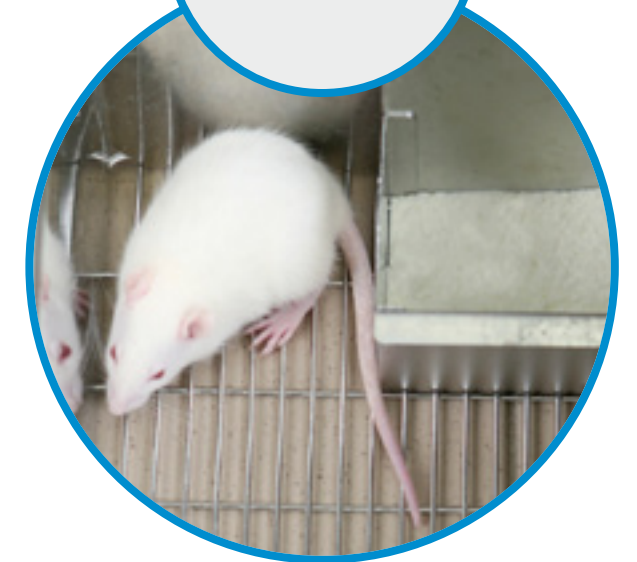
- Department of Biological Function of Food - www.pan.olsztyn.pl/zbzf;
- Department of Immunology and Food Microbiology - www.pan.olsztyn.pl/zimz;
- Beneficiaries of the Welcome Project - welcome.pan.olsztyn.pl.

All experiments are conducted with the permission of the Local Ethical Committee for Animal Experiments in Olsztyn.



**animal laboratory, rats, mice,
food experiments, in vivo**

Contact
Przemysław Zduńczyk,
M.Sc.
p.zdunczyk@pan.olsztyn.pl



The Welcome Project Team



Principal Investigator
Leslie P. Kozak, Prof.

Post-doctoral fellows
Emilia Gospodarska, Ph.D.
Julia Jarosławska, Ph.D.

Ph.D. candidates
Marika Olga Ziętak, M.Sc.
Magdalena Jura, M.Sc.
Paweł Nowialis, M.Sc.
CHU DINH TOI, M.Sc.
Agnieszka Chabowska, M.Sc.

Laboratory Manager
Agnieszka Korytko

Research Associate
Elżbieta Malinowska

Student worker
Anna Trąbczyńska

The Project

Welcome Project: Nutrition and ambient temperature during early development can reduce susceptibility to obesity.

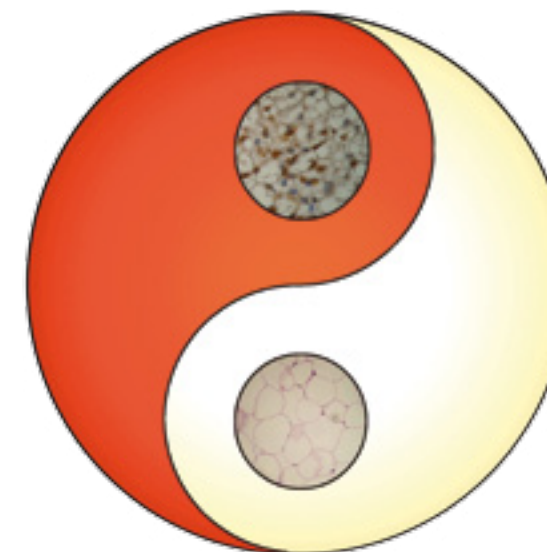
The energy balance equation explains with a simple formula the idea that energy intake must be in balance with energy expenditure to maintain a stable body weight. Because the energy balance equation is based upon the fundamental laws of thermodynamics, we know with scientific certainty that a life style and/or genetic constitution that leads to a positive energy balance, which occurs when energy intake is greater than energy expenditure, will lead to obesity. However, we do not know the structural-functional properties of our genetic constitution nor the interactions between our genes and environmental conditions that will lead to a positive energy balance. The realization of our genetic potentiality begins in the germ cells of our parents and continues through development in utero and during the post-natal suckling period. The goal of this research project is to determine the genetic and environmental conditions that affect one critical system of energy balance, that is, the adipose system.

Birth instantly presents the newborn mammal with 2 life determining challenges, acquisition of caloric energy by nursing and the maintenance of body temperature. Adipose tissue plays a vital and necessary role in both of these physiological processes. How these challenges are met has life-long consequences on energy balance in adults. As powerfully described in an epidemiological study of adult men who were at critical stages of fetal or post-natal development during the Dutch Famine in the winter of 1945 [1], malnutrition during early development can either



stimulate or suppress susceptibility to obesity in adults. Accordingly, a major challenge lies in determining the molecular and cellular process affected by the nutritional environment during early development that will determine the capacity for adipose tissue expansion. Acquisition and management of caloric energy and maintenance of body temperature depend on white and brown adipocytes. White adipocytes have the

biochemical mechanisms to store fat in lipid vesicles and then release it to the system when energy stores are low, whereas the reciprocal function of brown fat is to produce heat to maintain body temperature. Do the interconnected, opposing functions of brown and white adipocytes, symbolized by the ying-yang motif, have long term effects on energy balance of an individual? Accordingly, the Kozak laboratory seeks to determine how changes in energy balance as a consequence of variation in environmental temperature and diet during early post-natal development establishes the cellular balance between white and brown adipocytes.



**Welcome program in obesity
and type 2 diabetes**

Research

Welcome Project

During the nursing period of post-natal development two independent developmental processes determine the state of adipose tissue, one is a hard-wired developmental process known as adipogenesis, the other is a process dependent upon two variable environmental conditions, one is the nutritional state and the other is the ambient temperature. Variation in nutrition and temperature continually determine the state of energy balance. Using a mouse model in which the caloric intake of the dam is manipulated during post-natal development, it has been established that the nutritional environment during the nurs-



ing period determines the capacity for adipose tissue development by 10 days of age. This capacity is a genetically independent fixed phenotypic trait that maintains its effect on the physiology of an individual into adulthood. Knowing how strongly the ambient temperature affects adiposity in adult mice, Prof. Kozak and his team are now exploring the consequences of a low ambient temperature on the early development of both brown (BAT) and white (WAT) adipose tissues. The clinical significance of temperature is obvious from the epidemic of childhood obesity in Southern USA (<http://www.cdc.gov/HealthyYouth/obesity>), the burst in childhood obesity in Europe in general, but especially in the southern/Mediterranean regions (International Obesity Task Force, London 2002), and the recent realization that adult humans have depots of brown adi-



pose tissue. This has led to the development of the present hypothesis: **An imbalance in nutrition and chronic high ambient temperatures during early development are major environmental determinants of childhood obesity.**

UCP-1 and alternative thermogenesis

Mice in which the Ucp1 gene has been inactivated by genetic engineering has established the importance of the UCP1 protein in maintaining body temperature by the production of heat. While one might be tempted to say that this specific genetic experiment has established that UCP1 is necessary (and maybe sufficient) for the production of heat to protect body temperature; there are exceptional phenotypes reminding us that the physiological dynamics of heat production are much more varied and interesting than simply adrenergic activation of brown fat thermogenesis. The appearance of novel thermodynamic phenotypes in fact owe their discovery to the targeted inactivation of Ucp1 by homologous recombination. What are some of these thermogenic phenotypes that require resolutions.

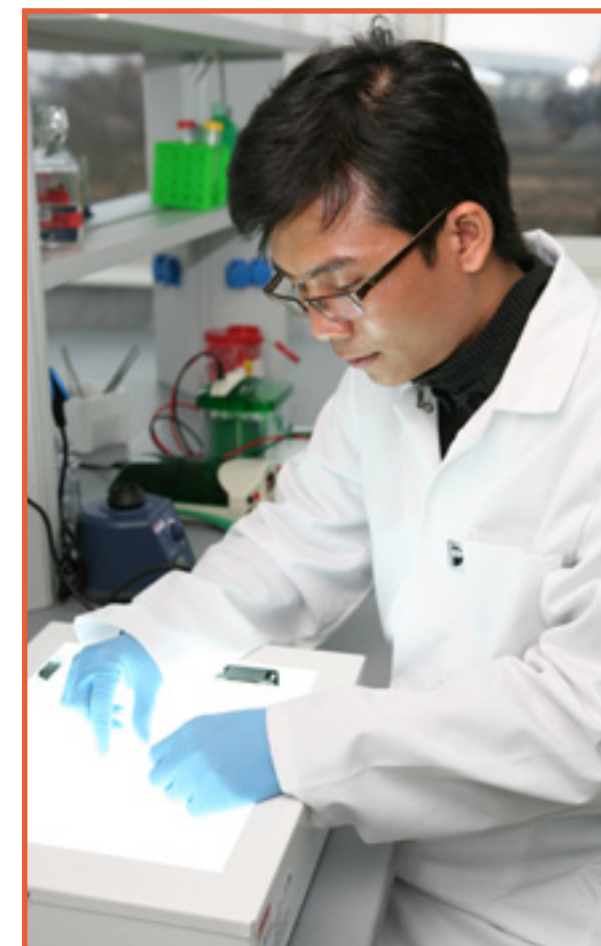
Analytical facilities

1. Olympus IX51 inverted microscope with camera;
2. Olympus BX 43 light microscope with camera and fluorescence attachment;
3. Ultralow freezers;

4. Eppendorf 5417R refrigerated microcentrifuge with rotors;
5. Eppendorf 5804R refrigerated clinical centrifuge;
6. Electrophoresis/immunoblot apparatus;
7. Eppendorf Mastercycler Pro PCR machines;
8. Environmental incubators for mice;
9. Bruker nuclear magnetic resonance for determining body composition.

Selected papers

1. Kozak L.P., Kozak R.A., Anunciado-Kozak R., Mendoza T., Newman S. (2012) Inherent plasticity of brown adipogenesis in white fat of mice allows for the recovery from the effects of post-natal malnutrition. PLoS ONE 7 (2): e30392.
2. Kozak L.P. (2012) The effects of early undernutrition on the development of wBAT and obesity. A commentary on the Inherent Plasticity of Brown Adipogenesis in White Fat of Mice Allows for Recovery from Effects of Post-natal Malnutrition, Adipocyte.
3. Kozak L.P. (2011) The genetics of brown adipocyte induction in white fat depots. Frontiers in Endocrinology 2: 1-13.
4. Gawronska-Kozak B. (2011) Scarless skin wound healing in FOXN1 deficient (nude) mice is associated with distinctive matrix metalloproteinase expression. Matrix Biol. 30 (4): 290-300.



Contact
Leslie P. Kozak, Prof.
l.kozak@pan.olsztyn.pl

International partners

- Dr. Anthony Vidal-Puig, Institute of Metabolic Science, University of Cambridge, USA;
- Dr. Sven Enerback, Institute of Medical Biochemistry, Göteborg University, Sweden;
- Dr. Indu Kheterpal, Albemarle Corporation, USA;
- Dr. Robert Koza, Pennington Biomedical Research Center; USA;
- Dr. Michael Salbaum, Laboratory for Regulation of Gene Expression, Pennington Biomedical Research Center/Louisiana State University, USA.

Institute in recent international projects

<p>Development of transduction layers for construction of biosensors used in food quality control, medical diagnostics and natural environmental monitoring.</p> <p>Coordinator: Prof. Jerzy Radecki Project: Poland - Belgium 679/N-Belgium/2010/0 Realization: 2010-2013</p>	<p>Studies on flavonoids and their metabolites diffusion through brain borders in the context of their preventive action on neurodegenerative changes.</p> <p>Coordinator: Prof. Mariusz K. Piskuła Project No.: 798/N-COST/2010/0 Realization: 2010-2012</p>
<p>Whey protein hydrolysates as potential modulators of the intestinal ecosystem in the aspect of obesity.</p> <p>Coordinator: Dr. Dominika Świątecka Project No.: Dec – 2011/01/M/NZ9/06836/COST Realization: 2011-2014</p>	<p>Effect of embryo-maternal interactions on creating immunotolerance in the environment of the uterus in pigs.</p> <p>Coordinator: Dr. Marek Bogacki Project No.: 738/N-COST/1010/0 Realization: 2010-2012</p>
<p>The role of cytokines and morphogenes in the regulation of embryo-maternal interactions during the peri-implantation period in pigs.</p> <p>Coordinator: Dr. Agnieszka Blitek Project No.: 717/N-COST/2010/0 Realization: 2010-2012</p>	<p>Influence of ovarian steroids and selected pro-inflammatory cytokines on the production and release of prostaglandin in equine uterus – <i>in vitro</i> study.</p> <p>Coordinator: Prof. Dariusz Skarżyński Project No.: DPN N5/COST/2010 Realization: 2010-2012</p>
<p>Isolation of stem cells from porcine uterus. Models useful in clinical xenotransplantation, organ cultures, uterine receptivity and embryo implantation.</p> <p>Coordinator: Dr. Gabriel Bodek Project No. 796/N-COST/2010/0 Realization: 2010-2012</p>	<p>Unlocking potential of the Institute of Animal Reproduction and Food Research for reinforced ERA integration and regional development – REFRESH.</p> <p>Coordinator: Prof. Mariusz K. Piskuła Project No.: FP7- REGPOT-2010-1-264103 Realization: 2010-2014</p>

Publication co-financed by the REFRESH Project



"Unlocking potential of the Institute of Animal Reproduction and Food Research for reinforced ERA integration and regional development"



Research Potential - Capacities - 7th Framework Programme
EU FP7-REGPOT-2010-1-264103





Institute of Animal Reproduction and Food Research
of the Polish Academy of Sciences

10 Tuwima Street, 10-748 Olsztyn
tel. (+48 89) 523 46 86
fax (+48 89) 524 01 24
e-mail: institute@pan.olsztyn.pl
www.pan.olsztyn.pl