Japanese-Polish Joint Seminar

"Cutting-edge Reproductive Physiology –

Key processes for birth of

a new life"

Sunday, September 8th

18:00-20:00 - Welcome reception & Registration – Atos Hotel restaurant

Monday, September 9th

8:00-8:45 - Registration

8:45-9:00 - Opening ceremony - Kyoshi OKUDA & Dariusz SKARŻYŃSKI & Tomasz JANOWSKI

9:00-9:45 - **Plenary lecture I –** ***Tolerance and Innate Immunity Shape the Development of Endometritis*****, Martin SHELDON**

9:45-10:00 - Coffee break

10:00-12:00 **Session I: Endometritis and Endometrosis and Placentitis in mares.** Moderators: Ch. AURICH; I. CANISSO

10:00-10:30 - *Update on diagnostic procedures and therapeutic approaches for endometritis in the horse*; Christine AURICH,

10:30-11:00 - *Recent developments in the pathogenesis, diagnosis, and treatment of placentitis in mares*; Igor F. CANISSO,

11.00-11:20 - *Changes in secretion of anti-inflammatory cytokines and acute-phase proteins in the uterus after artificial insemination in the mare*; Roland KOZDROWSKI

11:20-11:40 - *Does epigenetics regulate MMP2 and MMP9 transcripts in equine endometrosis?*; Graca FERREIRA - DIAS

11:40-12:00 - *The role of mediators of inflammation in the development of mare endometrosis*; Anna SZÓSTEK – MIODUCHOWSKA

12:00-12:10 - *Comparative study of steroids receptor expression in mare endometrial fibroblast and myofibroblast culture*; Natalia LECIEJEWSKA

12:10-13:00 Lunch

13:00-13:45 Plenary lecture II - *Bovine endometritis and bacteriology of uterus*, Takeshi OSAWA

13:45-14:00 Coffee break

14:00-16:00 Session II: Endometritis in cows. Moderators: M. DRILICH; R. de La SOTA

14:00-14:30 - *Current concepts of endometritis in cattle*; Marc DRILICH

14:30-15:00 - *Metritis and endometritis in grazing dairy cows: risk factors, diagnosis, treatment and reproductive performance*; Rodolfo Luzbel de La SOTA

15:00-15:20 - *Influence of intrauterine administration of Lactobacillus buchneri DSM 32407 on reproductive performance and pro-inflammatory endometrial mRNA expression of cows with subclinical endometritis*; Christoph GABLER

15:20-15:40 - *Inhibin, folliculostatin and peristatin as new markers for uterine health and future pregnancy*; Dawid TOBOLSKI

15:40-16:00 - *The effect of clinical endometritis on the ovarian activity in high yielding dairy cows during post-partum period*; Zeravan MOHAMMED

16:00-16:15 Coffee break

16:15-17:35 Session II: Endometritis in cows. Moderators: M. DRILICH; R. de La SOTA

16.15-16.35 - *Cytological endometritis in primiparous and multiparous dairy cows -an analytic approach for diagnosis based on endometrial polymorphonuclear cells cytology threshold*; Tal RAZ

16:35-16:50 - *Uterine bacterial metagenomics and bacterial load in dairy cows with metritis and cytological endometritis*; Ron SICSIC

16.50-17:05 - *Exploring the role of Streptococcus uberis in bovine endometritis*; Panagiotis BALLAS

17:05-17:20 - *Investigation of the reproductive tract health in cows with different degrees of pneumovagina*; E. Sinem ÖZDEMİR SALCI

17:20-17:35 - *Interleukin 8 mRNA was higher expressed in the endometrium at the time of artificial insemination in cows that did not conceive compared with their fertile counterparts*; Karen WAGENER

20:00-23:30 - **Gala Dinner - Stary Dom Restaurant**

Tuesday, September 10th

8:30-9:00 - Registration

Endometritis and Polish-Japanese Seminar JOINT SESSION

9:00-9:45 **Plenary lecture III -** *MicroRNAs: small but potent molecules in animal reproduction,* **Monika KACZMAREK**

9:45-10:15 Coffee break

10:15-12:30 **Session III: Immune-endocrine function of uterus.** Moderators: J. SCHÖN; B. JALALI

10:15-10:45 - *Current approaches and future perspectives for modeling the female reproductive tract in vitro*; Jennifer SCHÖN

10:45-11:05 - *Remodeling of porcine endometrium during Peri-implantation period: molecular changes*; Beenu MOZA JALALI

11:05-11:25 - *Understanding the uterine environment in subfertile cattle*; Shuichi MATSUYAMA

11:25-11:45 - *The influence of Heat Stress on the Endocrine Function of Bovine Endometrium*; Koji KIMURA

11:45-12:00 - *Heat stress alters innate immune responses in bovine endometrial cell*; Sunsuke SAKAI

12:00-12:15 - *Bisphenol a and its analogs affects contractile activity of the porcine uterine smooth muscle*; Aleksandra ZYGMUNTOWICZ

12:15-12:30 - *Obesity Alters Leptin Signalling in Mouse Uterus: Putative Link to Epigenetic Regulation During Decidualisation*; Edyta WALEWSKA

12:30-13:30 Lunch

Polish-Japanese Seminar: Cutting edge of Reproductive Physiology - Key processes for birth of a new life

13:30-14:55 OVARIAN FUNCTIONS – FOLLICLE/CORPUS LUTEUM. **Moderators: A. BLITEK, R. NISHIMURA**

13:35-13.55 - *Role of prostacyclin in the corpus luteum of the pig*; Agnieszka BLITEK

13.55-14:15 - *Role of glycoconjugates and lectins in the corpus luteum*; Junko NIO-KOBAYASHI

14:15-14:35 - *VASPIN – new adipokine in the ovarian physiology: expression and direct effect*

on signaling pathways, steroidogenesis, proliferation and apoptosis. In vitro studies on the porcine model; Agnieszka RAK

14:35-14:55 - *Multiple roles of hypoxia in bovine corpus luteum function*; Ryo NISHIMURA

14:55-15:25 Coffee break

15:25-16:30 OVARIAN FUNCTIONS – CORPUS LUTEUM. Moderators: A. BLITEK, R.NISHIMURA

15:25-15:45 - *The action of transcription factor Gata4 on gonadal promoter regulation is modulated via PKA and ERK1/2 pathway in steroidogenic cells*; Hiroaki TANIGUCHI

15:45-16:05 - *The effect of PGF2α on synthesis and release of progesterone through peroxisome proliferator-activated receptors in the bovine corpus luteum*; Barbara SOCHA

16:05-16:30 - *Progesterone receptor isoforms in bovine corpus luteum*; Robert RĘKAWIECKI

Wednesday, September 11th

9:00-11:00 OVIDUCT FUNCTIONS and GAMETS AND EARLY EMBRYO DEVELOPMENT. Moderators: Y. YAMAMOTO; A. ANDRONOWSKA

09:00-09:20 - *Generating mechanisms for spntanieus rhythmic contraction of bovine oviduct*; Yuki YAMAMOTO

09:20-09:40 - *The effect of lysophosphatidic acid (LPA) on ovarian follicle function, oocyte fertilization and early embryo development in cows*; Izabela WOCŁAWEK – POTOCKA

09:40-10:00 - *Multi-step process of ciliogenesis in bovine oviductal epithelium during the estrous cycle*; Sayaka ITO

10:00-10:20 - *Function of the porcine oviduct under different physiological and hormonal conditions*; Aneta ANDRONOWSKA

10:20-10:40 - *The novel techniques of in vitro maturation of porcine oocytes using 3D culture systems*; Gabriela GORCZYCA

10:40-11:00 - *The biology of telomeres and TERRA in bovine oocytes- two aspects of reproductive ageing*; Paweł KORDOWITZKI

11:00-11:30 Coffee break

11:30-12:30 CURRENT ASPECTS OF HORSE BREEDING IN POLAND AND JAPAN AND WILD ANIMALS. Moderators: K. OKUDA; D. SKARŻYŃSKI

11:30-11:50 - *The history and current view of polish konik horse*; Marta SIEMIENIUCH

11:50-12:10 - *Status and prospect of Horse breeding and reproductive treatment in Japan*; Yasuo NAMBO

12:10-12:30 - *The relationship between size of sebaceous glands in back skin and plasma testosterone concentration in male brown bear*; Jumpei TOMIYASU

12:30-12:40 Closing ceremony - Kyoshi OKUDA & Dariusz SKARŻYŃSKI & Tomasz JANOWSKI

12:40 Lunch

The Conference is organized under funds of PAS: joint JSPS-PAS project, BWZ and DUN.

Greeting

We are very happy to participate again in the joint seminar between Poland and Japan. This is the fourth seminar of “Cutting-edge Reproductive Physiology”, in which Polish and Japanese reproductive scientists gather. The first seminar was held in Krakow in 2005 with a theme “Regulation of ovarian function”. The second was held in 2011 in Morioka/Japan “From gamete to baby” and the third was in 2015 in Gdansk “Path to pregnancy: Regulation mechanisms at watershed point”. All the seminars have been supported by the Polish Academy of Sciences and the Japan Society for the Promotion of Science. Joint research between the two countries in reproductive science has intensively been promoted by the exchange of many researchers from 20 years ago. Although the collaborative researches have mainly been promoted on the ovarian and uterine functions in cattle, We are very happy that it has spread to a wide range of fields such as researches about gametes, fertilization as well as development of embryos in many species.

It goes without saying that the exchange of scientific information is an important purpose of this seminar, but the most important one is to expand collaborative research between Poland and Japan. We hope that many attendants of both countries will discuss about new collaborative projects during the sessions and find a new partner to carry out some collaborative researches.

Kiyoshi OKUDA

Dariusz J. SKARZYNSKI

Speakers

Kiyoshi OKUDA, Japanese Organizer of the Joint Seminar

Kiyoshi OKUDA, DVM, Dr.med.vet., Ph.D., is the President of Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. His research focuses on mechanisms in regulating bovine corpus luteum function as well as uterine and oviductal function. His team has been studying local regulati1on of luteolysis including molecular mechanisms of luteal cell death, uterine PGF2α secretion in cattle. They found that a variety of local factors including cytokines as TNFα plays big roles not only in regulating luteolysis but also in uterine PG synthesis. They have also demonstrated that a hypoxic condition, which is induced by bleeding at ovulation and by reduced blood flow at the late luteal stage, leads to luteal development by stimulating hypoxia inducing factor-1 (HIF) resulting in VEGF production, as well as functional and structural luteolysis. In addition, the recent studies of his team are focusing on the regulation of bovine oviductal function, especially local and systemic factors in regulating the contraction of oviduct and ciliary movement. Recently, they showed that PGF2α auto-amplification system in endometrium plays a big role to induce and complete the corpus luteum regression in the mare. Many of his team's published papers are collaborative studies with Polish groups.

Dariusz J. SKARZYNSKI, Polish Organizer of the Joint Seminar

Dariusz J. SKARZYNSKI (DVM, Ph.D,, Dr.Sci) is a Head of Department of Reproductive Immunology and Pathology and Scientific Director of the Institute of Animal Reproduction and Food Research of PAS, Olsztyn. His current research focuses on three subjects: (1) endocrinology and immunology of reproduction, especially immunoendocrine, cellular and molecular regulations of the release and action of cytokines and arachidonic acid metabolites in the female reproductive tract; (2) Immuno-endocrine mechanisms controlling embryo-maternal interactions in large animals and humans as well mechanisms leading to the early embryo mortality; (3) regulation of angiogenesis, apoptosis and necroptosis in the female reproductive tract; (4) experimental *in vivo* and *in vitro* models for the study of early embryo mortality and endometrial fibrosis. He carried out research as visiting fellow/professor at: 1) Okayama University, Japan (postdoctoral fellow of the JSPS; 1997-1999, visiting professor, 2003-2013, in total 16 months); 3) Faculty of Veterinary Medicine, University in Lisbon, Portugal (visiting professor, 2008-2019); 4) Interdepartmental Centre for Studies on Biology of Reproduction, University of Siena, Siena, Italy (visiting professor, 2012-2016); 5) Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, Israel (visiting professor, 2013). He was a member

of the Council of the National Centre for Research and Development (the head of Commission for special objectives of NCR&B, 2010 -2014) and currently is the coordinator at IRZBZ PAN in Olsztyn of the project "Healthy Animal - Safe Food" within the National Scientific Lead Center (KNOW) in the field of veterinary science (2015-2019).

Takeshi OSAWA

Takeshi OSAWA, DVM, MSc, MPhil, PhD, is a Professor of Theriogenology at the Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan. After he qualified as a veterinarian in 1989, he worked in School of Veterinary Sciences, National University of Asuncion, Paraguay, as a teaching assistant as well as a large animal practitioner for two years. He worked as an Assistant Professor and Associate Professor at Laboratory of Theriogenology of Iwate University, Morioka, Japan, from 1998 until he took the current position in University of Miyazaki in 2012. During his career he received a MSc (Serotypes of *MAIS* complex in pigs) and a PhD (Role of endogenous opioid peptides around parturition in postpartum resumption of pituitary and ovarian functions in dairy cows) from Rakuno Gakuen University, Hokkaido, Japan, and MPhil (Development of an ELISA to detect antibodies to *Neospora caninum* in cattle, sheep and goats, and its use in epidemiological studies) from University of Edinburgh, Scotland, UK. Also he joined JICA projects in Uruguay, 2000-2001, and in Vietnam, 2004. He served as a visiting professor at Ontario Veterinary College, University of Guelph, Ontario, Canada, 2010. His research interests are development of diagnosis-treatment protocol for reproductive disorders including uterine diseases and reproductive management with timed AI program using ultrasonography to improve reproductive performance in postpartum dairy and beef cows.

Agnieszka BLITEK

Agnieszka BLITEK, Ph.D., is a Professor of Agricultural Sciences in the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland; head of the Department of Hormonal Action Mechanisms. The main scientific interests of the Department are mechanisms involved in the regulation of ovarian and uterine functions during the estrous cycle as well as embryo-uterine interactions during the peri-implantation period in the pig. Her research team has been studying (1) the effect of hormonal synchronization of estrus on the corpus luteum function and uterine preparation for implantation; (2) consequences of supplementation with exogenous progesterone on endometrial receptivity for implantation; (3) the expression and role of peroxisome proliferator-activated receptors in conceptuses and early placenta; and (4) prostacyclin synthesis and action in the endometrium, conceptuses, and corpus luteum of the pig. They showed that prostacyclin and its receptor

signaling system are an important component of the embryo-maternal cross-talk during early pregnancy. Moreover, they demonstrated that prostacyclin may directly promote luteotropic activity through upregulation of progesterone synthesis in the porcine corpus luteum.

Yasuo NAMBO

Professor Yasuo Nambo belongs to Global Agro-medicine Research Center, Obihiro University of Agriculture & Veterinary Medicine, and has been working as a specialist of equine reproduction. He majors in equine endocrinology, ultrasonography and related technology as well as general equine practice. Until 2014, he had worked as an official veterinarian as well as a scientist in Japan Racing Association (JRA) for 21 years. He graduated from Obihiro University in 1993, then got a PhD degree from The United Graduated School of Veterinary Science, Gifu in 1999. During his working at Hidaka Training & Research Center, JRA, he had published a lot of findings for more efficient breeding of Thoroughbreds. In 2010, he advanced to Chief, Equine Science Division in Hidaka Training & Research Center. In 2014, he moved to Department of Clinical Veterinary Science, Obihiro University, as a full professor. From 2016, he has also become a Chief in Section of Horse assisted activity in the University.
His current interests are 1) effect of extended photoperiod on various physiological functions in the mare and the stallion, 2) mechanisms of action on inhibin/activin, insulin like peptide 3, anti-mullerian hormone et al. in horses, 3) prediction of abortion and parturition using biochemical findings and ultrasound imaging, 4) diagnosing reproductive soundness and disorder by endocrinological test, and 5) frozen sperms Artificial Insemination and Embryo Transfer in Japanese native pony. Professor Nambo awarded Japanese Society of Equine Science, Society Award in 2014. So far, he published 120 papers in scientific journal.

Roland KOZDROWSKI

Roland Kozdrowski Ph.D., is the professor of theriogenology. He graduated Faculty of Veterinary Medicine at the Agricultural University in Wrocław, Poland in 1998. Next he started PhD studies at the Faculty of Veterinary Medicine in Wroclaw and obtained PhD degree in 2002. His research activity is focused on issues related to the physiology and pathology of reproduction (mainly on diagnosis of infertility), and on methods connected with assisted reproduction i.e.: artificial insemination, and improvement of protocols used for semen cryopreservation. He is also interested in wild animal reproduction but the main areas of his interests is etiopathogenesis, diagnosis and treatment of endometritis in the mare. His current research focuses on the diagnosis of chronic endometritis, including the role of proinflammatory cytokines in the pathogenesis of subclinical endometritis, and on the pathogenesis of persistent breeding-induced endometritis in mares.

Koji KIMURA

Koji KIMURA, Ph.D. is a professor of laboratory of Animal Reproductive Physiology, Graduate School of Environmental and Life Science at Okayama University, Okayama, Japan. He was a senior researcher at National Institute of Livestock and Grassland Science, Japan for more than 20 years and moved to Okayama University 5 years ago as an associate professor. His PhD and postdoctoral studies investigated sexual dimorphism in IFN-tau (the maternal recognition signal) production from bovine embryo in the University of Missouri, Columbia. Currently his research interest has expanded to combat the decreasing pregnancy rates of cattle, with a specific focus on identifying differences in endometrial function between fertile and sub-fertile cattle. Moreover, he also focuses on the uterine function under the heat stress condition, in which reproductive performance of cattle is severely deteriorated In this symposium, he will present his recent progress in the study of effect of heat stress on endocrine function in bovine endometrium.

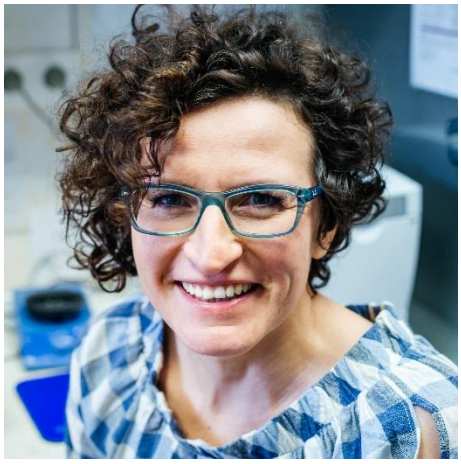
Izabela WOCŁAWEK-POTOCKA

Izabela Wocławek-Potocka, DVM, Dr.med.vet., Ph.D., graduated from Veterinary Medicine Faculty, University of Warmia and Masuria in Olsztyn in 2002. Currently she is the Professor of Animal Reproduction at the Institute of Animal Reproduction and Food Research of PAS, Olsztyn, Poland. Her research focuses on the examination of intracellular mechanisms controlling early embryo development during *in vitro* embryo production as well as embryo-maternal communication during pregnancy establishment in the cow. Her research team has been studying endocrine backgroud of the disturbance of gamete and embryo development in the cow, focusing especially on assisted reproduction techniques and early embryo mortality.

Junko NIO-KOBAYASHI

Junko NIO-KOBAYASHI, D.V.M., Ph.D., is the Lecturer of the Laboratory of Histology and Cytology of Graduate School of Medicine of Hokkaido University. She was graduated from the Faculty of Veterinary Medicine, Hokkaido University in 2002, and earned her Ph.D. in 2006 from the Graduate School of Veterinary Medicine, Hokkaido University. Her research interest is the role of glycoconjugates and lectins, sugar-binding proteins, in the regulation of various pathophysiological events in the human and animals. She has been investigated the role of galectin, a β-galactoside-binding lectin, in the regulation of the corpus luteum in mice and women. She has been collaborating with Prof. Kiyoshi Okuda, Obihiro University of Agriculture and Veterinary Medicine, and Prof. W. Colin Duncan, University of Edinburgh for the research in bovine and human corpus luteum. She is revealing that glycosylation, especially sialylation and glycosphingolipids, is important for the regulation of the function of the corpus luteum.

Monika M. KACZMAREK

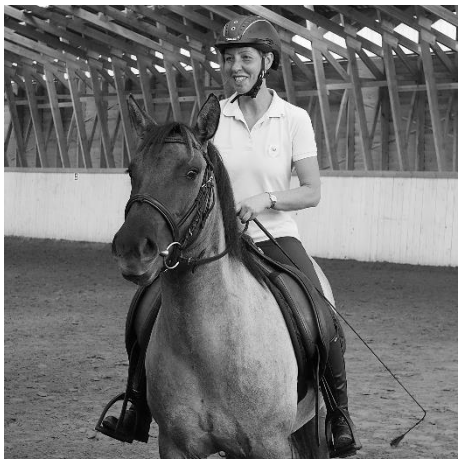


Monika M. KACZMAREK, Ph.D., is a professor at the Department of Hormonal Action Mechanisms in the Institute of Animal Reproduction and Food Research, Polish Academy of Science in Olsztyn, where she also leads Molecular Biology Core Facility. Her research interests are wide, but at the very most related to biology of reproduction. In her Ph.D., performed under the supervision of Prof. Adam J. Ziecik, she evaluated experimental treatment of mammary gland tumors. As the Alexander von Humboldt Foundation fellow at the Technical University of Munich she carried out studies on the role of angiogenesis in reproduction under the supe rvision of Prof. Dieter Schams. For almost a decade her research has been focused on untangling the role of non-coding RNAs in embryo-maternal dialog leading to successful pregnancy. In 2009-10 she visited Prof. Leslie P. Kozak laboratory at Pennington Biomedical Research Center, Louisiana State University in Baton Rouge under the umbrella of the Fulbright Program. Since then her interests evolved towards understanding the mechanisms of nutritional programming of reproductive performance over generations. During her scientific carrier, she was awarded several scientific fellowships and research grants and has provided a broad range of services to the scientific community as well as governmental and professional organizations.

Shuichi MATSUYAMA

Shuichi Matsuyama, Ph.D., is an Associate Professor at the Laboratory of Animal Production Science, Nagoya University, Nagoya, Japan. He is interested in the cause of subfertility in cattle. His research focuses on endometrial function in subfertile cattle. He and his colleagues have demonstrated that some mitochondrial dysfunctions associated with cellular senescence would be occurred in the endometrium of subfertile cattle and they might contribute to infertility. Currently, based on the idea that a failure of uterine repair after parturition causes mitochondrial dysfunctions in the endometrium, he and his colleagues are trying to unravel the mechanism of endometrial regeneration after parturition in cattle.

Marta SIEMIENIUCH



Marta Siemieniuch, Ph.D. in Veterinary Sciences, is an Associate Professor of the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland. Research area involves physiology and pathology of equine reproduction, particularly equine endometritis, endocrinology of reproduction, regulation of the estrous cycle, equine subfertility, ethological mechanisms in free-ranging horses. In 2006 she was employed in the Department of Reproductive Immunology and Pathology of the IAR&FR PAS in Olsztyn. From 1st July 2016 she head an equine husbandry in Research Station in Popielno belongs to IAR&FR where is localized the reservoir and farm breeding of Polish Konik Horses. Scientific achievements were distinguished with numerous prizes. So far, she has directed 4 research projects financed from external sources and participated as the executor in 4 national projects (2P06K 025 29; 2P06K 003 30; Ministry of Science and Higher Education N308 327 933; National Science Centre 2011/01/B/NZ5/04173) and 2 international (Agreement between the Republic of Poland and the Republic of Portugal on scientific and technical cooperation in the project no 7: „Apoptosis and angiogenesis in the ovary of mare during the estrous cycle" 2007-2009 and international project not co-financed by the Ministry of Science and Higher Education as part of the action COST FA0702, No DPN N5/COST/2010: "Influence of ovarian steroids and proinflamatory cytokines on prostaglandin secretion in equine endometrium: an *in vitro* model". She participated in numerous scientific internships in national and international centres (Balice, Japan, Switzerland, Portugal) including 12-month post-doc internship at Okayama University, Japan (2008-2009). Besides conducting of the research work, she is a member of the Polish Konik horses Association, which belongs to the Polish Horse Breeders Association.

Ryo NISHIMURA

Ryo NISHIMURA, Ph.D., is an Assistant Professor at the Laboratory of Theriogenology and Veterinary Medical Center, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan. He obtained his Ph.D. degree in Okayama University in 2006, defending his thesis entitled "Studies on hypoxia as an important factor for inducing luteolysis in cattle", under supervision of Professor Kiyoshi OKUDA. He specializes in reproductive physiology, especially in corpus luteum function in the cow. His current research is focusing on the roles of hypoxia and its related signals induced by hypoxia-inducible factor (HIF) in luteal formation and regression in the cow as a research supported by Grant-in-Aid for Young Scientists of the Japan Society for the Promotion of Science (JSPS). His recent study showed that hypoxia stimulated a glucose metabolism by inducing glucose transporter 1 (GLUT1) in the early stage CL, suggesting the importance of glucose uptake for luteal formation in the cow.

Aneta ANDRONOWSKA

Aneta Andronowska, Ph.D., D.Sc. is an Associate Professor in Deptartment of Hormonal Action Mechanisms in Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences. She obtained a PhD (in Agricultural Science; speciality: animal husbandry, endocrinology of reproduction) with honours from IARFR PAS. Her research has been focused on reproductive physiology. Her research focused on immuno-endocrine and molecular mechanisms involved in: embryo-uterine interactions during maternal recognition of pregnancy and implantation; endometrial remodelling (effect of cytokines/chemokines on tissue and cell remodelling); estrus synchronization and its effect on oviduct function, as well as the influence of immune system on corpus luteum function. She has been a visiting fellow at Academic Department of Obstetrics and Gynecology, University Birmingham in UK, Instituto de Nutrition y Bromatologia C.S.I.C. Universitaria in Madrid in Spain, University Medicine Greifswald in Germany, Department of Physiology, Institute of Biomedicine, Faculty of Medicine, University of Turku in Finland and Institute of Biomedicine and Translational Medicine, Department of Pathophysiology, University of Tartu in Estonia. She has been elected to Management Committee COST Action 16119 CellFit "In vitro 3-D total cell guidance and fitness".

Agnieszka RAK

Agnieszka RAK, Ph.D., is an Assistant Professor at Department of Physiology and Toxicology of Reproduction, Faculty of Biology, Jagiellonian University in Kraków. Her research focuses on mechanisms that controlling female reproductive processes by energy homeostasis and enviromental pollutions. Her research team reported that the expression of ghrelin and adipokines like resistin, vaspin or apelin and their receptors mRNAs and proteins in the porcine ovarian follicular and luteal cells depends on the phase of the oestrous cycle and, thus, seems to be associated with hormonal status of animals. It has also reported that the metabolic hormones regulate ovarian steroidogenesis, signalling pathways, proliferation and apoptosis. Recently, they investigate about the relationships between adipokines and the human placenta cells physiology including proliferatiin, cell cycle and endocrine function. Dr Rak was also involved in a research on the effect on enviromental pollutions *e.g.* polycyclic aromatic hydrocarbons, bisphenol A, polybrominated diphenylethers, polychlorinated naphthalenes or pesticides on ovarian and placenta cell function. Author and co-author 3 scientific chapters books, more than 60 scientific papers and more than 90 conference abstracts.

Beenu MOZA-JALALI

Beenu Moza-Jalali Ph. D., is Adjunct Professor in the Department of Reproductive Immunology and Pathology at Institute of Animal Reproduction and Food Research, Olsztyn where she has been a faculty member since 2010. She completed Ph.D at Jamia Millia Islamia University, New Delhi, India and her Ph.D thesis was based on addressing protein folding problems with a thrust on understanding protein stability, kinetics and characterizing folding intermediates. After being awarded a Ph.D degree in 2003, she was a postdoctoral fellow at Boston Biomedical Research Institute, Watertown, MA, from 2004 till 2007. Her research there involved development of antagonists for superantigen mediated diseases and this work generated a patent related to development of „Neutralizing agents" for bacterial toxins. Since 2010, she is involved in understanding mechanisms resulting in successful establishment of pregnancy. Her current research mainly concerns role of immune cells and their cytokine products in blastocyst adhesion and implantation, endometrial remodeling to generate receptive endometrium for successful pregnancy and mechanisms associated with corpus luteum formation and development.

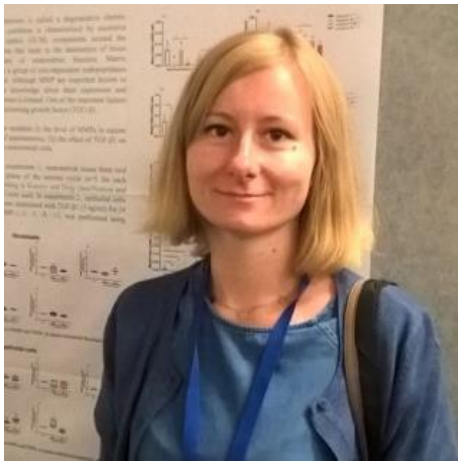
Robert RĘKAWIECKI

Robert Rękawiecki Ph.D. Assistant Professor at Department of Physiology and Toxicology of Reproduction in Institute of Animal Reproduction and Food Research of Polish Academy of Sciences. He graduated Faculty of Biology at Nicolaus Copernicus University in Torun, Poland in 1998. In the same year he started working in Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Poland on a position of Assistant in Department of Physiology and Toxicology of Reproduction where he obtained the PhD degree in 2004. His research concerned on isoforms of nuclear progesterone receptor in the reproductive system of the cow. He was a grant manager of three research grants related to this topic. He completed an internship in Vienna Biocenter Core Facilities in Vienna, Austria in Department of Bioinformatics & Scientific Computing in the subject of preparation, implementation and analysis of next generation sequencing data. In 2018 he graduated D.Sc. (habilitation) of agricultural sciences in Institute of Animal Reproduction and Food Research of Polish Academy of Sciences. Current topic of his research focuses on the methylation promoters of progesterone receptor isoforms in the regulation of the reproductive function of the cow.

Yuki YAMAMOTO

Yuki YAMAMOTO, D.V.M., Ph.D., is an Assistant Professor in Laboratory of Reproductive Physiology, Department of Animal Science, Division of Agricultural and Life Science, Graduate School of Environmental and Life Science, Okayama University in Japan (since 2012). She graduated Tokyo University of Agriculture and Technology in 2008 and obtained a Ph.D. degree from Gifu University in 2011. After that, she worked in Lab. of Veterinary Physiology, Tokyo University of Agriculture and Technology during 2011-2012 as a postdoctoral research fellow. Her current research focuses on the regulatory mechanisms of oviductal function in cow. Oviduct provides the optimal environment for fertilization, early embryonic development and transport of gametes and embryo. Her research group investigates the function of epithelial, stromal and smooth muscle cells using molecular biology methods. The objectives of her group are to clarify the 1) regulatory mechanism of transport of gametes and embryo and 2) mechanism of environmental control for fertilization and early embryonic development in the oviduct.

Anna SZÓSTEK-MIODUCHOWSKA



Anna Szóstek-Mioduchowska Ph.D., is an Assistant Professor in Department of Reproductive Immunology and Pathology in Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences. She graduated Faculty of Biology at University of Warmia and Mazury in Olsztyn, Poland in 2008. Next, she started PhD studies in Department of Reproductive Immunology and Pathology in Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn. She obtained her PhD degree in 2012. She had post-doc position in Laboratory of Reproductive Endocrinology Graduate School of Natural Science and Technology in Okayama University in Japan; under the Japan Society for Promotion of Sciences from 2014 to 2015. Her researches focused mainly on issues related to the physiological and pathological processes taking place in endometrium in mare. Her current research focuses on the role of mediators of inflammation in the development of equine endometrosis.

Changes in secretion of anti-inflammatory cytokines and acute-phase proteins in the uterus after artificial insemination in the mare

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The mechanisms responsible for development of persistent breeding-induced endometritis (PBIE) are not fully understood. The objective of the study was: 1) evaluation of the concentrations of anti-inflammatory cytokines: interleukin-1 receptor antagonist (IL-1RA) and interleukin-10 (IL-10) in uterine lavage fluid before and after artificial insemination (AI); 2) evaluation of the concentrations of acute-phase proteins: serum amyloid A (SAA) and haptoglobin (Hp) in uterine lavage fluid before and after AI; and 3) determination of the importance of fluid accumulation in the uterine lumen during estrus in the etiopathogenesis of PBIE. Based on ultrasound examination mares were divided into three groups: group 1 (n=9) served as a control group. In this group, no fluid was detected in the uterus during estrus and 7 h after AI. In group 2 (n=8), no fluid was detected in the uterus during estrus but 7 h after AI a depth of more than 2 cm of fluid was detected in the uterus. In group 3 (n=8), more than 2 cm of fluid was detected in the uterus during estrus and also 7 h after AI. In all groups of mares 7 h after AI a significant increase in polymorphonuclear cells (PMN) was recorded, however, no differences were found among particular groups of mares before and after AI. The concentration of IL-1RA before AI was significantly higher in group 3 mares compared to group 1, while the concentration of SAA before AI was significantly lower in group 1 compared to group 2. After AI, a significant increase in IL-1RA and SAA was observed in all three groups of mares, however no significant differences were detected among the groups of mares at this time. No differences were found in the concentration of IL-10 and Hp among particular groups of mares before and after AI. Modulation of the inflammatory process in response to AI is a part of the innate immune defense mechanism and based on our results we suggest that inflammatory response and anti-inflammatory processes occurring 7 h after semen deposition are similar in mares resistant and susceptible to PBIE. At this time, independent from the status of the mare before AI, the endometrial response characterized by PMN influx, and SAA, Hp, IL-1RA and IL-10 production, is similar. The presence of intrauterine fluid during estrus is not connected with PMN influx but can impact uterine IL-1RA production at this time, and also can reduce pregnancy rates.

The role of mediators of inflammation in the development of mare endometrosis

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Endometrosis is a degenerative chronic condition in the equine endometrium, defined as an active or inactive fibrosis around the endometrial glands and/or in the stroma, often associated with pathological changes in the endometrial glands within fibrotic foci [1-2]. This condition is characterized by excessive deposition of extracellular matrix (ECM) components, as collagen type I (COL1) and fibronectin around the endometrial glands and stroma that leads to the destruction of tissue architecture and impairment of endometrial function [1-3]. Equine endometrosis causes not only alterations in the uterine morphology, but also in functions, ultimately leading to changes in uterine microenvironment, ovarian cycle and early pregnancy dysfunction [1; 3; 5-7]. The pathogenesis of equine endometrosis is not yet well known. However, it has been hypothesized that this condition occurs as a consequence of repeated, chronic inflammation due to insults associated with breeding, foaling and veterinary intervention [1; 8-9]. Inflammation seems to be associated with development of fibrosis in a paracrine way by the secretion of profibrotic chemokines, cytokines and other factors from injured tissue and inflammatory cells. Inflammatory mediators could act on endometrial, as well immune resident cells, such as fibroblasts, monocytes and macrophages, and affect fibrogenesis and ECM remodeling [10-11]. This process could include quantitative and qualitative changes in the ECM, mediated by enzymes that are responsible for ECM degradation, such as matrix metallopeptidase (MMPs). Their activity is low in physiological conditions but elevated during repair or remodeling processes [12]. Our study showed that the level of MMP-1, -2, -9 was up-regulated in the course of endometrosis compared to category I endometrium in follicular phase of estrous cycle ($P<0.05$). In turn, the level of MMP-3 was down-regulated in category III endometrium compared to category 1 endometrium in mid luteal phase of estrous cycle ($P<0.05$). Our findings suggest that in endometrial degeneration the level of endometrial MMPs and TIMPs is altered. Although MMP are important factors in the process of fibrosis, the knowledge about their regulation in equine endometrosis is still limited. The aim of our study was to investigated the effect of prostaglandin (PG)E_2, $PGF_{2\alpha}$, interleukin (IL)-1β, IL-6 and transforming growth factor (TGF)-β1 on MMPs and their inhibitors as well as on COL1 and COL3 in equine endometrial cells and tissue. Prostaglandin E_2 treatment increased MMP-2 and MMP-9 secretion, and decreased MMP-13 secretion in a time and dose-dependent manner in equine endometrial fibroblasts ($P<0.05$). In turn, $PGF_{2\alpha}$ treatment increased MMP-2, MMP-13 and COL1 secretion and decreased MMP-1 secretion in time and dose-dependent in equine endometrial fibroblasts ($P<0.05$). IL-1β treatment up-regulated secretion of COL1, MMP-2,

TIMP1, and TIMP2 in category I endometrial fibrosis tissues (P<0.05). IL-6 treatment up-regulated secretion of ECM, MMP-2, and MMP-3 and down-regulated secretion of MMP-9 in category I endometrium (P<0.05). Transforming growth factor-β1 up-regulated MMP-1, -3, -9, TIMP-2, COL1 and COL3 secretion but down-regulated MMP-3 secretion from fibroblast cells in time-dependent manner (P<0.05). In epithelial cells, TGF-β1 up-regulated MMP-1, -9, -13 and TIMP-1, -2 in time-dependent manner (P<0.05). Our results showed that PGs, ILs and TGF-β1regulated expression of MMPs and their inhibitors (TIMPs) and COL what strengthen the hypothesis that mediators of inflammation participate in development of mare endometrosis by effect on ECM remodeling.

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Bovine endometritis and bacteriology of uterus

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Heavy bacterial colonization in the genital tract subsequent to trauma, dystocia, or poor hygiene and poor uterine defense mechanisms can lead to establishment of puerperal uterine infection. In cattle, nonpathogenic bacteria in the uterus disappear more quickly after a difficult calving than after normal parturition, and pathogenic isolates persist longer in dystocia-affected animals. Although prevalence of subclinical endometritis in beef cows is usually much lower than dairy cows, suckled cows had higher percentage of neutrophils in the endometrium (PMN%) than early weaned cows during early postpartum (pp) period in Japanese Black cows [1]. Poor nutritious status that resulted from energy consumption and fat mobilization by suckling may be involved in the delay not only in the resumption of ovarian cyclicity but also restoration of the endometrium in the process of uterine involution.

Escherichia coli and *Trueperella pyogenes* are known to be pathogenic bacteria causing endometritis. In our study, pathogenic bacterial detection rates were higher in the cows with endometritis at week 3 pp (W3) and W5 than those at W7 [2]. We analyzed blood metabolite concentrations with persistent bacterial uterine infection, that caused by *T. pyogenes* and anaerobic bacteria, uterine bacteriological swabs were collected from lactating Holstein cows at W5 and W7, and PMN% was evaluated [3]. Glucose concentrations prepartum were negatively correlated with persistent bacterial infection postpartum ($P < 0.01$). Decreased prepartum blood glucose concentrations might be an important risk factor for persistent pp uterine infection. We also investigated the relationship between the persistence of uterine bacterial infections with endometritis and ovarian function [4]. A positive correlation ($P < 0.001$) was noted between the severity of endometritis and the persistence of infection. Cows with persistent infections had a significantly prolonged luteal phase compared with cows without infection ($P < 0.01$).

Mycoplasma and ureaplasma species are believed to be not part of the normal vaginal flora. *M. bovigenitalium* and *U. diversum* have been detected in the genital tract of normal and repeat breeder cows, which has led to speculation concerning its role as a pathogen [5,6]. We investigated the incidence of mycoplasma infection in the uterus of pp dairy cows and its relationship to the occurrence of endometritis. Intrauterine samples of the dairy cows at W5 and W7 were placed in mycoplasma culture broth as well as specific agar plates and cultured. A rapid PCR was used to detect seven mycoplasma species. Of the seven mycoplasma species, only *M. bovigenitalium* was detected in 7.4% of the samples. The incidence of dystocia was higher ($P < 0.001$) in mycoplasma positive (29%) compared with mycoplasma negative (2%) cows. In addition, the incidence of cytological endometritis was higher ($P < 0.05$) in mycoplasma positive (50%) than mycoplasma negative (24%) cows at W7. *M. bovigenitalium* infection in the uterus may be associated with recent dystocia and with endometritis in pp dairy cows.

Recent studies have reported that *Lactobacillus* spp. are present in the uterus of the cow during uterine restoration period in pp. Co-culture experiment with *L. ruminis* and endometrial

epithelial cells *in vitro* confirmed that *Lactobacillus* spp. not causing endometrial epithelial cells damage, conversely, revealing immunomodulatory properties [7]. We evaluated the influence of *Lactobacillus* spp. on uterine environment in pp dairy cows. The results suggested that *Lactobacillus* spp. reduce PMN% in cows with endometritis, may inhibit the growth of *T. pyogenes*, and accelerate the clearance in the uterus in the medium stage of pp. Conversely, absence of *Lactobacillus* spp. with low PMN% during the last stage of pp implies that uterus is completely involuted. Therefore, timing of the presence of *Lactobacillus* spp. during pp may have a diagnostic significance. *Lactobacillus* spp. can improve health status of the reproductive tract and help accelerate uterine involution in pp dairy cows.

In an attempt to eliminate endometrial pathogens, bovine practitioners have used antibiotics. However, the drawbacks are residues in meat and milk and potential development of bacterial resistance. Therefore, it is important to find effective therapies without such negative consequences. Use of probiotics such as *Lactobacillus* spp. or other type of antimicrobial agents such as povidone-iodine may be an option for therapeutic or preventive measures to control bovine endometritis.

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MicroRNAs: small but potent molecules in animal reproduction

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MicroRNAs (miRNAs) are small, approximately ~22-nt, non-coding, endogenous and mostly evolutionarily conserved RNAs that mediate post-transcriptional gene repression [1]. These molecules control a wide range of biological processes, including metabolism, cell proliferation, apoptosis, and differentiation in almost all cell types across the animal kingdom. Today, a total of 2654 mature miRNAs have been identified in *Homo sapiens* (miRBase: Release 22.1: Oct 2018). In other animals, numbers of mature miRNAs were identified, *e.g.*, 1978 in *Mus musculus*, 1025 in *Bos taurus*, and 457 in *Sus scrofa*. Bound with an Argonaute protein to form a silencing complex (RISC), miRNAs function as sequence-specific guides, directing the silencing complex to transcript, primarily through Watson-Crick pairing between the miRNA seed sequence (nucleotides 2–7) and complementary sites located preferentially within the 3′untranslated regions (3′UTRs) of target RNAs [2,3]. The miRNAs conserved to fish have been grouped into 87 families, each with a unique seed region. On average, each of these families has hundreds of conserved targeting interactions, and together these interactions involve most mammalian mRNAs [4]. Interestingly, a large proportion of miRNAs are localized as clusters in the genome, transcribed together from physically adjacent miRNAs and show similar expression profiles [5].

To date, hundreds or even thousands of molecules have been investigated in mammals during crucial reproductive events, but their function still remains unknown in most of the cases. Recently, miRNAs have been suggested to play an important role in control of oocyte maturation, folliculogenesis, corpus luteum function, implantation, and early embryonic development. For example, knockout of Dicer, the cytoplasmic enzyme that cleaves the pre-miRNA to its mature form, results in early embryonic lethality in several animal models, *e.g.*, mouse [6]. miRNAs were shown to be involved in processes associated with establishment and maintenance of pregnancy, including preparation of the *endometrium* for implantation [7] or control of genes linked to inflammatory responses [8]. Studies performed in our laboratory indicated that both the conceptus and the *endometrium* can be a source of miRNAs involved in the embryo-maternal dialogue during early pregnancy in pigs [9,10]. Our recent results have shown that unique sets of miRNAs can already be observed in circulation of pigs during the first weeks of pregnancy, as a result of initiated embryo-maternal communication [11]. In addition, miRNAs are currently being examined as potent modulators of cell-to-cell communication at the embryo-maternal interface, as well as events supporting conceptus development and maintenance of luteal function during early pregnancy in the pig.

Collectively, it has become clear that miRNAs serve as key regulators of gene expression, and our understanding of their functional relevance in reproductive events is exponentially growing. However, much work remains to be done to understand the precise roles of miRNA-mRNA interactions in mammalian reproduction.

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Remodeling of porcine endometrium during peri-implantation period: molecular changes

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Implantation and establishment of pregnancy in mammals, including pigs, require effective interactions between a competent blastocyst and the receptive uterus. Towards this goal, uterine endometrium during the peri-implantation period undergoes morphological and molecular transformations to support the embryo development and implantation. These changes, called "plasma membrane transformations" are induced by the ovarian steroids and later modified by the conceptus-secreted factors to regulate the 1) para-cellular permeability across the epithelium and 2) increase the adhesion between trophoblast and endometrial epithelial cells. The plasma membrane transformations during early pregnancy period are associated with a partial loss in polarity of epithelial cells that is regulated by tight junction, adherens junction, polarity protein complexes and actin binding proteins.

Using techniques such as qPCR, western blotting and immunofluorescence, we present the evidence that, in pigs, depending upon the stage of estrous cycle and reproductive status of the animals (between Days 10-16 of cycle and oregnancy), the endometrial epithelial cells undergo change in the expression or distribution of i) tight junction (TJ) proteins such as occludin (OCLN), claudin1 (CLDN1) and zona occludens-1 (ZO-1), ii) adherens junction (AJ) proteins cadherin (CDH1) and β-catenin (CTNNB), iii) partition defective (PAR) complex - PAR/PAR6/aPKC/CDC42 proteins and iv) actin binding proteins such as cofilin (CFL), vinculin (VCL) and gelsolin (GSN).

The TJ proteins OCLN and CLDN1 were concentrated on the lateral plasma membrane in pregnant endometrium between Days 13 and 16 as compared to corresponding days of cycle. On the contrary, ZO-1 abundance was decreased in endometrium of pregnant animals on Day 16 with it translocation to the basal side that results in change in epithelial polarity. A strong reactivity of AJ proteins, CDH1 and CTNNB was observed in the lateral plasma membrane of pregnant animals during implantation period. As observed by co-localization and co-immunoprecipitation results, CDH1 and CTNNB associated with each other in lateral plasma membrane only on Day 16 of cycle and pregnancy. We speculate that there is a possibility that lateral CDH1 distribution and its interaction with CTNNB during implantation period might be a possible way adopted by porcine endometrium to limit attachment of conceptus trophoblast to the apical epithelium. The PAR complex proteins were expressed in the porcine endometrium with little to no change in their expression during days of estrous cycle or pregnancy. However, a significant decrease in *PAR6* expression was observed on Day 16 of pregnancy as compared to corresponding day of estrous cycle. On Day 16 of pregnancy, whereas, a redistribution of aPKC to lateral plasma membrane from its apical position on Days 10 and 13 was observed, CDC42 protein was more concentrated towards the apical pole of luminal epithelium on Days 13 and 16 of pregnancy.

The tight junction proteins are known to interact with the actin cytoskeleton. Along with the changes in the distribution of junction and polarity proteins, we observed that among the

actin binding proteins investigated; VCL abundance was significantly higher in Day 16 of pregnant endometrium as compared to other days of cycle of pregnancy. A further investigation of VCL function in cell adhesion revealed that it facilitates conceptus attachment to endometrial epithelial cells through focal adhesion kinase.

In summary, porcine endometrial epithelium undergoes transformations during peri-implantation period that alters its paracellular permeability to allow the transport of molecules from uterine stroma to the conceptuses and change the adhesive property of epithelial cells to facilitate trophoblast adhesion.

Understanding the uterine environment in subfertile cattle

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Reproductive failure in an individual is caused by various external and internal factors. Moreover, the coexistence of multiple elements makes it difficult to delineate the effects of each individual factor on reproductive function. Previous studies have shown that fertilization rates following artificial insemination (AI) of cattle exceed 90% [1], suggesting that embryonic death is responsible for the majority of reproductive failures. Several findings indicate that most embryonic deaths occur before and just after the pregnancy recognition period. Previously, we showed that embryos transferred into the uterus died immediately, prior to reaching the pregnancy recognition period [2], suggesting the uterine environment during embryo transfer strongly influences embryo viability. The gene expression pattern in the endometrium of cattle has been associated with an optimal uterine environment. Forde et al. revealed that circulating progesterone concentrations in the first few days after estrus may influence the endometrial gene expressions in cyclic heifers [3] and, ultimately, the ability of the uterus to support conceptus development. Therefore, we hypothesized that a functional disorder of the endometrium could lead to early embryonic death and infertility.

In our study, global gene expression analysis revealed that endometrial gene expression pattern was different between fertile and subfertile cattle. Interestingly, a larger number of genes were more highly expressed in subfertile cattle than in fertile cattle- genes encoding ribosomal proteins and mitochondrial oxidative phosphorylation in particular were remarkably highly expressed. These results indicated that cellular senescence was induced in the endometrium of subfertile cattle. Cellular senescence is characterized by irreversible cell cycle arrest and one of the cellular pathways contributing to aging. It has been reported that aging suppresses mitochondrial biogenesis and autophagy [4]. The age-related decline in autophagic activity might cause accumulation of damaged mitochondria [5], then it might lead organs dysfunctions. SIRT1 and Bcl2L13 are the genes which are involved in the regulation of mitochondrial biogenesis and mitophagy, respectively. Quantitative RT-PCR analysis demonstrated that SIRT1 and Bcl2L13 mRNA expressions were significantly lower in subfertile cattle than in fertile cattle. In addition, the mitochondrial DNA copy number was significantly higher, whereas the ATP content did not differ between fertile and subfertile cattle. Based on these results, the malfunctions of mitochondrial biogenesis and mitophagy would accumulate damaged mitochondria in the endometrium of subfertile cattle.

Resveratrol, a small polyphenolic antioxidant compound, has been shown to accelerate mitophagy [6]. In our study, Bcl2L13 mRNA expression slightly increased and the mitochondrial DNA copy number slightly decreased in subfertile cattle following resveratrol administration—each reaching a point between the values observed for fertile and subfertile cattle—though the effects were not significant. From these results, we speculate that resveratrol increased mitophagy in subfertile cattle, but not to the level seen in fertile cattle. Additionally,

two out of five subfertile animals treated with resveratrol—but none of the five subfertile cattle who received the vehicle—conceived after embryo transfer. Considering resveratrol's effect, it is possible that mitophagy malfunction in the endometrium is a cause of both an inadequate uterine environment for embryonic survival and subfertility in cattle. Collectively, some mitochondrial dysfunctions (i.e. mitophagy disorder) associated with cellular senescence would be occurred in the endometrium of subfertile cattle and they might contribute to infertility.

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The Influence of Heat Stress on the Endocrine Function of Bovine Endometrium

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The impact of global warming on the livestock industry is a critical issue, particularly that heat stress decreases fertility in cows. This decrease is caused by elevated body temperature which directly or indirectly affects hormone secretions from the hypothalamus and pituitary, ovarian function, estrous behavior, folliculogenesis, oocyte competence and embryonic development. Moreover, it has been indicated that heat stress also alters uterine function and, consequently, the environment for embryo development. The aims of the present study are to investigate the influence of heat stress on the endocrine function of bovine endometrial cells.

In the first experiment, the effects of heat stress on the production of prostaglandin (PG)E2 and PGF2α in cultured bovine endometrial epithelial and stromal cells were examined separately. Epithelial and stromal cells from endometrium derived from local slaughterhouses were enzymatically collected, and then cultured at 38.5℃(control) or 40.5℃ (HS). After treatment, PGE2 and PGF2α levels were measured by enzyme immunoassay (EIA) and mRNA expressions of enzymes involved in PG synthesis were examined via quantitative reverse transcription polymerase chain reaction. HS did not influence the production of PGE2 or PGF2α in the epithelial cells, however, HS significantly enhanced the production of both PGE2 and PGF2α in the stromal cells ($p < 0.05$). In addition, HS significantly increased *phospholipase A2 (PLA2), cyclooxygenase 2 (COX2), prostaglandin F synthase (PGFS), prostaglandin E synthase (PGES), and carbonyl reductase 1 (CBR1)* mRNA expression in the stromal cells ($p < 0.05$).

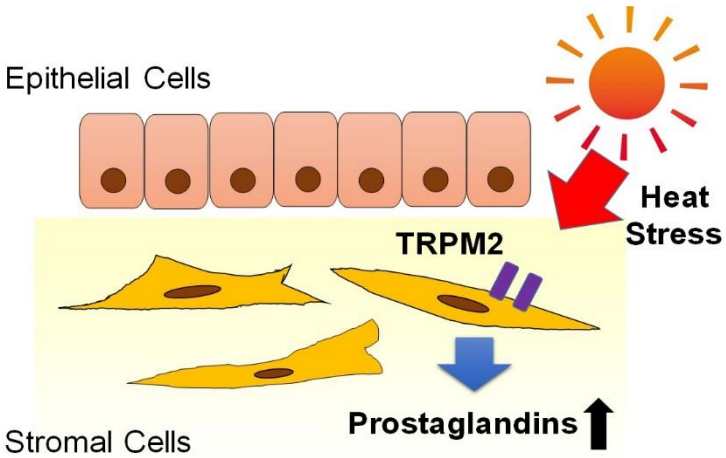

Figure 1. Mechanism of sensing and response to heat stress in bovine endometrium

Each cell in the body has several signaling pathways and cascades to sense and react to various environmental stimuli such as osmolality, temperature, and mechanical stimuli. Transient receptor potential (TRP) channels are ion channels located on plasma membranes, some of which are involved in temperature sensing. The second experiment was conducted to investigate the role of TRP channel-mediated temperature sensing on the enhancement of PG secretion from bovine endometrial cells under HS conditions. Firstly, we investigated the endometrial location of temperature-sensing TRP channels (TRPV3, V4, and M2), which can sense bovine body temperature, by immunohistochemistry. Slaughterhouse derived uteri

classified at the late luteal stage (day 14–17) were used for the experiment. Uterine horns ipsilateral to corpus luteum were fixed and sliced, and each section was immunostained with antibodies against TRPV3, TRPV4, or TRPM2.

While TRPV3 was not clearly observed in the bovine endometrium, TRPV4 was slightly detected in luminal epithelial cells. TRPM2 was observed in each region of the uterine tissues, especially in luminal and glandular epithelial cells, which presented stronger signals than stromal cells, myometrium, and endothelial cells.

Next we investigated the effect of TRP channels inhibition on PGs secretion by HS endometrial stromal cells. As mentioned above, HS did not influence PGs secretion by epithelial cells; therefore, only stromal cells were used in the present experiment. Bovine endometrial stromal cells were collected and cultured in the presence of each of the antagonists for TRPV3, V4, and M2 (n = 5, 5, and 9, respectively) for 34 hours, at either 38.5℃ (control) or 40.5℃ (HS). After incubation, the PGs concentrations in the culture media were measured by EIA. When stromal cells were cultured in the presence of TRPV3 or TRPV4 antagonists, HS significantly increased the secretion of PGs ($p < 0.05$) compared to the controls, suggesting that inhibition of TRPV3 and TRPV4 does not reduce the effect of HS on PG secretion by bovine stromal cells. On the contrary, when the TRPM2 antagonist was added to the culture medium, PGs secretion significantly decreased ($P < 0.05$) under HS condition. No significant difference was observed in the PGs secretion of HS and control at higher concentrations of TRPM2 antagonist. Moreover, this effect was not detected in controls.

Taken together, the present study suggests that bovine endometrial stromal cells can sense heat stress via TRP channels and then increase the production of prostaglandins (Figure 1).

Role of prostacyclin in the corpus luteum of the pig

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Corpus luteum (CL) is a transient endocrine gland that develops from the postovulatory follicle. The principal function of the CL is to produce progesterone (P4) that is necessary for the establishment and maintenance of pregnancy. In all mammalian species, the lifespan of the CL determines the length of the estrous cycle [1]. CL is a highly vascularized tissue; thus, local hemodynamic changes within the blood vessels play a pivotal role in the regulation of CL development and activity. Since prostacyclin (prostaglandin I2; PGI2) is a well-known modulator of a vascular function, its role in luteal tissue seems to be crucial. PGI2 is a derivative of arachidonic acid and the terminal enzyme in its biosynthesis is prostacyclin synthase (PTGIS). PTGIS enzyme is abundant in endothelial and smooth muscle cells, but is also present in other cell types. PGI2 is chemically unstable in biological fluids and rapidly undergoes spontaneous transformation to its inactive form, 6-keto PGF1α, which thus directly reflects PGI2 concentration [2]. In the vascular system, PGI2 is a potent vasodilator and inhibitor of platelet aggregation [3]. The biological actions of PGI2 are mediated by a membrane G-protein-coupled receptor (PTGIR), whose activation leads to increased cAMP formation. PGI2 may also signal *via* nuclear peroxisome proliferator-activated receptors (PPARs), and PPARD isoform has been identified as a biological target for endogenous PGI2. Several synthetic PGI2 analogs have been demonstrated to directly interact with both PTGIR and PPARD isoform [4].

To clarify the role of PGI2 in the porcine CL we analyzed profiles of 1) PTGIS mRNA and protein expression, 2) PGI2 metabolite concentration, and 3) PTGIR and PPARD expression in luteal tissue on days 2 to 16 of the estrous cycle and days 10 to 30 of pregnancy. Moreover, we examined the *in vitro* effect of PGI2 analogs on 4) P4 synthesis by luteal cells and 5) the proliferation and proangiogenic gene expression in endothelial cells of the pig CL.

In cyclic gilts, decreased *PTGIS* mRNA expression was detected on days 15-16 compared to days 2-4. In contrast to mRNA, PTGIS protein level gradually increased during the studied period of the estrous cycle and was greater on day 12 compared with days 2-4. In pregnant animals, greater concentration of *PTGIS* transcripts was observed on day 30 compared to days 12, 15-16, 21, and 25-26 and was accompanied by elevated PTGIS protein level. However, the content of 6-keto PGF1α did not change in the luteal tissue of both cyclic and pregnant animals. A transient decrease in *PTGIR* mRNA expression was observed on days 5-7 of the estrous cycle. PTGIR protein level did not vary in the luteal tissue of cyclic animals, but increased on days 21 to 30 compared to day 10 in pregnant gilts [5]. *PPARD* mRNA expression also showed dynamic changes in the CL of both cyclic and pregnant gilts (*M. Szymanska, A. Blitek, unpublished*). The treatment of cultured luteal cells with stable analogs of PGI2, iloprost and carbaprostacyclin, increased *HSD3B1* mRNA expression and P4 secretion. Moreover, the addition of PTGIR or PPARD antagonists abolished a PGI2-stimulated P4 synthesis in luteal cells indicating that activation of PGI2 receptors has a luteotropic action in the pig CL [5]. *In vitro* proliferation of endothelial cells was stimulated by iloprost and this effect was mediated through the activation of PI3K signaling pathway. Moreover, iloprost increased *VEGF* and

ANGPT1 mRNA expression in cultured endothelial cells (*M. Szymanska, A. Blitek, unpublished*).

Summarizing, we reported dynamic changes of PTGIS and PGI2 receptor expression in porcine CL during the estrous cycle and early pregnancy. Moreover, our results indicate a possible luteotropic role of PGI2 in the pig. The supportive action of PGI2 on the CL development and function includes the stimulatory effect on P4 synthesis by luteal cells and proangiogenic activity in endothelial cells.

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Role of glycoconjugates and lectins in the corpus luteum

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Glycosylation is important for the regulation of cell function including development, differentiation, apoptosis, and signal transduction. Lectins are sugar-binding proteins, and endogenous animal lectins are classified into more than 10 families according to the sugar-binding affinity. Galectins are β-galactoside-binding animal lectins and 15 members are identified in mammals so far. Galectins recognize *N*-acetyllactosamine (LacNAc; Galβ1-3/4GlcNAc) and broadly distribute in mammalian body, modulating various cell functions. In the female reproductive system, galectin-1 and galectin-3 are major subtypes and their expression changes during estrous/menstrual cycle. In the mouse ovary, the regressing corpus luteum (CL) expresses both galectins. In cows and women, galectin-1 is expressed in the healthy functional CL whereas the galectin-3 expression elevates in the old regressing CL, suggesting subtype-specific function of galectins. We have been investigating the role of galectins in the human CL, and found that α 2,6-sialylation and glycosphingolipid contents are important for the regulation of the CL function.

Both galectin-1 and galectin-3 can bind to LacNAc motif; however α2,6-sialylation of terminal galactose blocks the binding of galectin-1 (Fig. 1). When we analyzed the expression of a α 2,6-sialyltransferase (ST6GAL1) in the human CL, the expression of *ST6GAL1* was high in the regressing CL like galectin-3, and inhibited by luteotrophic human chorionic gonadotrophin (hCG) and prostaglandin E (PGE). α 2,6-sialic acids were accumulated to 3β-HSD-negative luteal cells, suggesting that an inhibition of galectin-1-sugar-binding by α 2,6-sialylation is involved in luteal regression. We propose that there is a "galectin switch" associating with the CL function.

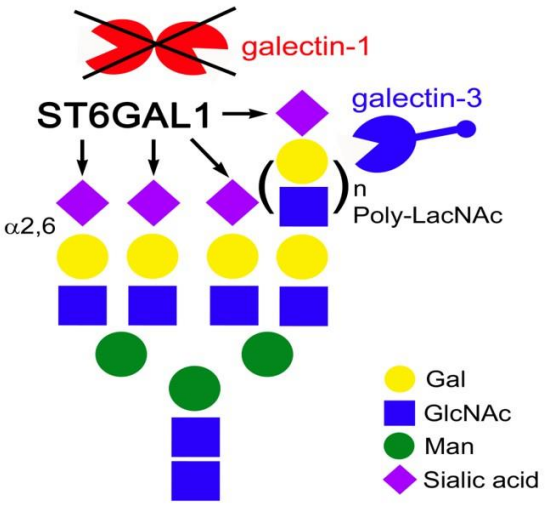

Fig. 1 Subtype specific sugar-binding affinity of galectin-1 and galectin-3. Both galectins bind to LacNAc motif. ST6GAL1 catalyzes α2,6-sialylation on terminal galactose (Gal) to block the binding of galectin-1. GlcNAc; *N*-asetylglucosamine, Man; mannose.

PGE is a major luteotrophic molecule and secreted from luteal cells by hCG/LH stimulation. Galectin-1 supports hCG/LH/PGE-activated cAMP/PKA signaling to maintain luteal function, and hCG/LH/PGE enhance the galectin-1 expression in luteal cells. However, the potential ability to produce PGE and reactivity against hCG/LH in luteal cells are attenuated with the age of the CL. Revealing how this phenomenon occurs is important for understanding the mechanism of spontaneous luteolysis in women because uterus-derived factors such as PGF are not involved in luteolysis in women.

We next focus on the glycosphingolipids in luteal cells and investigated how

glycosphingolipids regulate hCG signaling in human luteal cells. D-PDMP, an inhibitor of glycosphingolipid synthesis, decreased the basal and hCG-stimulated production of progesterone and PGE, suggesting that glycosphingolipids are important for luteal function in women. Ganglioside GM1 is sialic acid-containing glycosphingolipids and a major component of lipid raft, a membrane microdomain that is involved in signal transduction. Galectin-1 binds to ganglioside GM1 and probably supports hCG signaling by accumulating its receptor (LHCGR) on ganglioside GM1-rich lipid raft. Administration of ganglioside GM1 did not affect the luteotrophic action of hCG, whereas ganglioside GM3, the most simple ganglioside, attenuated the hCG-stimulated cAMP/PKA activation. Because the production of ganglioside GM3 is enhanced by luteal cell-derived steroids, it is likely that ganglioside GM3 is accumulated in luteal cell membrane with age of the CL, resulting in attenuation of cAMP/PKA signaling (Fig. 2). These results suggest that quantity and quality of glycosphingolipids are key factors to regulate the function of human luteal cells.

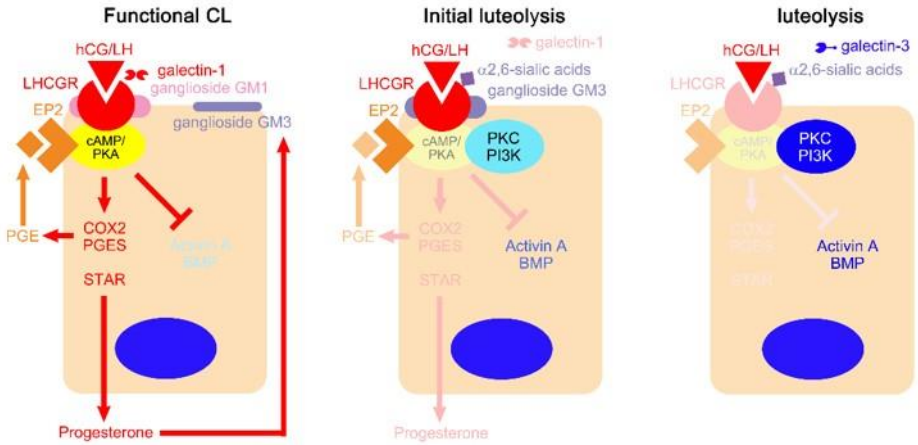

Fig. 2 Possible contribution of glycosphingolipids to spontaneous luteolysis in women. Accumulation of ganglioside GM3 attenuates cAMP/PKA signaling, resulting in an increase of a2,6-sialylation and luteolytic factors (Activin A and BMP). Finally, luteal cells totally lack the ability to produce progesterone and PGE as well as glycosphingolipids. Loss of glycosphingolipids results in change in signal transduction pathway towards PKC and PI3K. BMP; bone morphogenetic protein, COX2; cyclooxygenase 2, PGES; prostaglandin E synthase, STAR; steroidogenic acute regulatory protein.

VASPIN – new adipokine in the ovarian physiology: expression and direct effect on signaling pathways, steroidogenesis, proliferation and apoptosis. *In vitro* studies on the porcine model

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Introduction: The reproductive system in female is regulated precisely by an intricate interplay of hormones produced by the hypothalamus, pituitary and the ovaries. The interplay of hormones results in ovarian cyclicity in females, which in consequence leads to fertilization, delivery by the maintenance of pregnancy of offspring. Moreover, it is now clear that female fertility strong depends on the energy metabolism status in women and also domestic animals, including pig. For example, obesity and some metabolic disorders impairs women fertility through an effect upon the control of ovarian functions, ovulation, oocyte development, embryo and endometrial development, implantation and pregnancy loss [1]. Relevant energy status of sows determines the normal reproductive functions, *e.g.* sows with low weight require a longer time to first estrus and their offspring are less numerous and characterized by low birth weight [2, 3]. The metabolic function in the body is mediated, in part, by its ability to secrete numerous metabolic peptides like oxytocin, ghrelin, kisspeptin or adipose tissue hormones- adipokines: leptin, resistin, adiponectin, or visfatin, which modulate food intake, energy homeostasis, lipid and glucose metabolism, insulin resistance and also reproduction [4,5]. However, vaspin, new metabolic hormone, has never been studied in the ovary.

Aim of the study: Using porcine ovarian follicles, as a animal model, we studies: ***i).*** vaspin mRNA and protein expression, immunolocalization in the ovarian follicles; plasma and follicular fluid concentration of vaspin during oestrous cycle in two prolific breeds of pigs: fat Meishan (MS) and lean Large White (LW); ***ii).*** effect of several factors like gonadotropin, IGF1, insulin and steroids: P4- progesterone, T- testosterone, E2- estradiol) on ovarian vaspin expression; and understanding molecular mechanism involved by activation of different kinases: mitogen-activated protein kinases (MAPK/ERK1/2), Akt/phosphatidylinositol 3-kinase (Akt/PI3), Janus kinase (Stat/JAK2), 5'AMP-activated protein kinase (AMPKα) and nuclear factor kappa-light-chain-enhancer (NF-kB); ***iii).*** assessment of the putative direct action of vaspin on various kinases phosphorylation, steroidogenesis, proliferation and apoptosis..

Results: We found higher vaspin mRNA and protein expression in the ovarian follicles and adipose tissue at 10–12 days of the oestrous cycle in MS compared to LW. Moreover, vaspin expression, as well as its concentration in plasma and follicular fluid, decreased in ovarian follicles of LW during days of the oestrous cycle, while the opposite results were noted in MS. Immunohistochemistry showed vaspin in granulosa, theca, cumulus cells and oocytes as well as in adipocytes. Vaspin level in the ovary increased by gonadotropin, insulin, IGF-1 and steroids stimulation through kinases JAK/Stat, ERK1/2, PI3K and AMPK, as well as factor NF-κB. In the next part of our study, we observed that vaspin), in a time-dependent manner, increased phosphorylation of various ERK1/2, Akt, Stat3, AMPK α and PKA, while it decreased expression of NFKB2. We observed that vaspin, in a dose-dependent manner,

increased basal steroid hormones secretion (progesterone and estradiol), mRNA and protein expression of steroid enzymes and the mRNA of gonadotropins (FSHR, LHCGR) and steroids (PGR, ESR2) receptors. The stimulatory effect of vaspin on basal steroidogenesis was reversed when ovarian cells were cultured in the presence of a PKA pharmacological inhibitor (KT5720) and when GRP78 receptor was knocked down (siRNA). Finally, our *in vitro* studies documented that vaspin have stimulatory effect on cell proliferation and inhibitory on cellular apoptosis – related protein expression.
Conclusion: These findings all show vaspin expression and function in the ovarian cells, and provide the physiological framework to bring new insights of vaspin in the regulation of female reproduction.
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Multiple roles of hypoxia in bovine corpus luteum function

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Introduction

Development of the corpus luteum (CL) occurs after ovulation, and is accompanied by active angiogenesis. When conception does not occur, the CL regresses with the decrease of progesterone (P4) synthesis, accompanied by the apoptosis of luteal cells. During the ovarian cycle, blood flow to the ovary changes [1], causing changes in the transport of nutrients, hormones and gases including O_2 to the ovary. Ovarian blood flow in cows has been reported to decrease during luteal regression, and to be kept at low levels during luteal formation after ovulation [1] (Fig. 1). Thus, during luteal regression and formation, the ovary is characterized by a low oxygen condition (hypoxia) caused by the decreased blood supply. Cellular responses to hypoxic conditions are strongly influenced by hypoxia-inducible factors (HIFs) [2]. HIFs are hypoxia-specific transcription factors that have roles in inducing several physiological processes including angiogenesis and glycolysis. Our research has been focusing on the roles of hypoxic conditions in the regulation of ovarian function, with emphasis on luteal life and death.

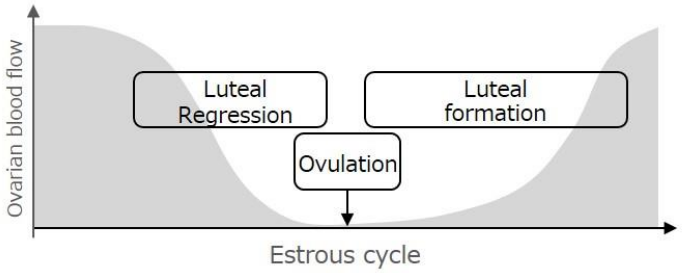

Fig. 1. Changes in ovarian blood flow to the ovary during the estrous cycle in cows.

Hypoxia in functional luteolysis

Luteal regression is characterized by a decrease in P4 production (functional luteolysis), followed by a decrease in luteal size (structural luteolysis), which is largely achieved by apoptosis. Since the decrease of blood flow during luteal regression occurs in parallel with the decrease of serum P4 level in cows [1], the decrease of blood flow has been suggested to be related to functional luteolysis. However, it remains unclear how the decrease of blood flow induces luteolysis. We hypothesized that oxygen deficiency is related to luteolysis. In experiments using cultured bovine mid luteal cells, P4 production decreased under hypoxia (3% O_2). The expression and activity of a cytochrome P450 side-chain cleavage enzyme (P450scc, CYP11A1), which converts pregnenolone into P4, were also decreased by hypoxia. These findings suggest that hypoxia has a role in functional luteolysis in cows.

Hypoxia in structural luteolysis

Apoptosis is an essential part of structural luteolysis [3]. We investigated whether hypoxia is related to structural luteolysis in cows by examining its effect on the viability of bovine cultured mid luteal cells and the integrity of their nuclei. We found that hypoxia induced

luteal cell death and DNA fragmentation. We concluded that apoptosis was induced in cultured mid luteal cells under hypoxic conditions. The expressions of caspase-3, an effector caspase in the apoptosis cascade, and Bcl-2 nineteen-kilodalton interacting protein 3 (BNIP3), which facilitates apoptosis and mitophagy under hypoxic conditions, were induced by hypoxia. The above findings suggest that the oxygen deficiency in the CL, which is caused by a decreased blood supply to the ovary, is one of the factors that accelerate structural luteolysis.

Hypoxia in luteal formation

Vascular endothelial growth factor (VEGF) is known to induce angiogenesis during luteal formation in cows, and its transcription is strongly induced by HIF1 [4]. The early luteal tissue just after ovulation is thought to be under hypoxic conditions, because of the destruction of the vasculature by ovulation. Therefore, we tested the hypothesis that luteal angiogenesis after ovulation is induced by hypoxia. In bovine CL, HIF1A protein expression was higher at the early and developing luteal stages than at the other luteal stages. Hypoxic conditions induced the protein expressions of HIF1A and VEGF in cultured bovine developing luteal cells. HIF1 is known to induce the expressions of genes for mitophagy (BNIP3) and glycolysis (glucose transporter 1; GLUT1). Our results showed that the expressions of BNIP3 and GLUT1 were high at the early luteal stage, and were up-regulated under hypoxia in early luteal cells. These findings suggest that hypoxic conditions caused by decreased blood supply and degraded vasculature immediately after ovulation are needed to form the new CL, and that activation of HIF1 and its downstream signals has a major role in this process.

Conclusion

The overall findings suggest that hypoxia plays multiple roles in both the formation and regression of the CL. Hypoxia induces angiogenesis during formation of the CL, and decreases P4 synthesis and promotes apoptosis during its regression. (summarized in Fig. 2). Further studies on how the length and degree of hypoxia control the fate of cells in each luteal stage and what other factors, such as hormones, regulate HIF1 signals will contribute to a better understanding of the roles of hypoxia in ovarian physiology.

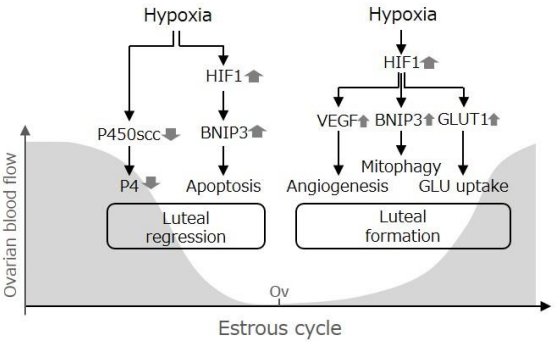

Fig. 2. Multiple roles of hypoxia in the formation and regression of the CL. HIF1: hypoxia-inducible factor 1, P4: progesterone, P450scc: cytochrome P450 side-chain cleavage enzyme, BNIP3: Bcl-2 nineteen-kilodalton interacting protein 3, VEGF: vascular endothelial growth factor, GLUT1: glucose transporter 1, GLU: glucose, Ov: ovulation.

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Progesterone receptor isoforms in bovine corpus luteum

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Progesterone (P4) is a steroid hormone that is produced by the corpus luteum (CL), placenta and ovarian follicle. The physiological effects of this hormone on target cells are achieved by binding to a specific nuclear progesterone receptor (PGR) belonging to the transcription factor-dependent receptor family. The PGR has two main isoforms, isoform A (PGRA) and isoform B (PGRB). The whole PGRA sequence is common to the PGRB sequence. In addition, PGRB has an additional sequence that distinguishes it from PGRA.

Our research, with using degenerated primers, revealed than in cow PGR also occur in two isoforms. A 429-nucleotide DNA sequence of the PGRB isoform was obtained. This sequence showed 79% similarity to the pig and horse sequence, 75% to the human sequence, 71% to the rat sequence and 68% to the mouse sequence [1].

Further research showed variable levels of mRNA and protein levels of PGRA and PGRB isoforms in CL during the estrous cycle. The highest level of PGRA and PGRB mRNA was found at the beginning of the estrous cycle, and thereafter it was gradually decreased to the end of the cycle. The high mRNA and protein levels of both isoforms at the beginning of the estrous cycle suggested their involvement in P4 regulation in newly formed CL. A comparison of the mRNA and protein expression profiles of the PGRA and PGRB isoforms revealed a 500-2000-fold lower mRNA concentration of PGRB during the cow cycle. In contrast, the difference in protein levels ranged from 2-8-fold in favor of PGRA. Isoform PGRA works as an inhibitor of PGRB. Therefore, the dominant mRNA and protein expression of PGRA in relation to PGRB may indicate that this isoform is a regulatory element of P4 activity, protecting CL against the possible effects of the overproduction of this hormone [1].

The mRNA and protein level of PGRA and PGRB isoforms were differently regulated by luteotropic and luteolytic factors in bovine endometrium. This may suggest that these factors through different impact on the mRNA and protein levels of PGRA and PGRB isoforms may regulate the effect of P4 in endometrial cells [2].

We found also that the most popular PGR antagonists as onapristone (ZK299) and mifepristone (RU486) affected the mRNA and protein expression of the PGRA and PGRB isoforms. This action was depended on the concentration of the given inhibitor. Obtained results may signify that the final physiological effect induced by the antagonist depends on the isoform of PGR that is associated with the compound [3].

The last step of PGR receptor activation is the attachment of additional transcriptional regulators called coagulators. This group consists coactivators, activating the transcription of target genes, and corepressors, inhibiting the transcription of such genes. We found that mRNA and protein level for P300/CBP-associated factor (PCAF) coactivator and the nuclear receptor corepressor 1 (NCOR1) in cow CL were the highest in the middle of the ovarian cycle in comparison to the levels at the beginning and the end of the cycle. In contrast in endometrium, the mRNA and protein levels NCOR1 and PCAF were higher at the beginning of the estrous cycle and decreased slightly to the end of the estrous cycle. The results showed also that the

mRNA and protein levels of PCAF and NCOR1 were corelated with the P4 level in both investigated tissues. This may suggest that P4 is involved in the regulation of their expression. While similar levels of coregulator and corepressor expression in CL and endometrium may denote that they compete for a binding site located in the PGR receptor [4].

Our latest research concerned the participation of methylation of PGR isoform promoters in the regulation of the PGR action. We showed that the methylation percentage of the PGRA promoter was approximately 2.5-3 times higher in CL, while in the endometrium it approximately 0.7-2.5 times higher than the methylation of the PGRB promoter during the estrous cycle. Conversely, the total methylation level of both isoforms in CL was approximately 2-fold higher than in the endometrium. These data could indicate that the increased methylation of the PGRA isoform promoter may be a regulatory mechanism of the inhibitory activity of PGRA against PGRB and thus influences the regulation of P4 activity in CL and endometrium [5].

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Generating mechanisms for spontaneous rhythmic contraction of bovine oviducts

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Spontaneous phasic contraction of mammalian oviducts occurs at peri-ovulation and is essential for the transport of gametes and embryos. Our previous studies revealed the regulatory mechanisms of contraction modulator production by bovine oviductal epithelial and stromal cells. However, knowledge of the system that generates spontaneous contraction of oviducts remains unclear. In the intestinal and urinary tracts, it has been demonstrated that spontaneous membrane depolarization, induced by inward or outward flow of several ions, increases intracellular Ca^{2+} levels in smooth muscle cells (SMCs) and results in spontaneous phasic contraction. In addition, ion currents also regulate pacemaker cells, which are endogenous generators of myocyte contraction. Based on these findings, our present study aimed to clarify the fundamental mechanisms underlying spontaneous contractions in bovine oviducts.

First, to clarify which ion channels are involved in the spontaneous rhythmic contraction of bovine oviducts, we investigated the effects of ion channel and gap junction blockers on contraction of oviduct strips using the Magnus system. The targets were as follows: (1) voltage-dependent Ca^{2+} channels (VDCC) and the receptors responsible for Ca^{2+} release from the endoplasmic reticulum—inositol trisphosphate receptor (IP3R) and ryanodine receptor (RyR); (2) Na^{+} and Cl^{-} channels that act as depolarization initiators; (3) voltage-gated potassium channels (VGKC) and Ca^{2+}-activated potassium channels (BK and SK channels) that act as re-/hyper-polarization regulators; and (4) gap junctions that mediate propagation of depolarization to neighboring cells. For these targets, we found that: (1) the VDCC blocker decreased the amplitude of contractions, resulting in loss of contraction. Co-inhibition of both IP3R and RyR decreased the number and amplitude of contractions. These results suggest that Ca^{2+} influx via VDCC, or release from the endoplasmic reticulum, is necessary for spontaneous contractions; (2) the Na^{+} channel blocker did not affect contractions, whereas Cl^{-} blockers decreased the frequency of contractions. This suggests that Cl^{-} channels are involved in the initiation of depolarization; (3) a VGKC blocker decreased the number and amplitude of contractions. BK channel blocking decreased the number of contractions, although BK and SK channel blockers increased the amplitude of contractions. These results suggest that VGKC and Ca^{2+}-activated K^{+} channels are involved in regulation of re-/hyper-polarization. (4) A gap junction blocker suppressed contractions, suggesting that depolarization propagates via gap junctions.

Next, intracellular Ca^{2+} imaging was performed in cultured bovine SMCs to evaluate the precontractile cellular activity. Intracellular Ca^{2+} was visualized by Fluo4. Movies using Ca^{2+} imaging were recorded and analyzed. When the area covered by smooth muscle actin-positive cells exceeded 90% (90% confluence), quick spontaneous Ca^{2+} oscillation was observed, but only a few slow Ca^{2+} oscillations were observed at 70% confluence. This indicates that spontaneous activity of SMCs can be observed in culture, and that cell-to-cell contact is required for the initiation of Ca^{2+} oscillations. We also found two types of Ca^{2+} signaling

patterns: Ca^{2+} waves and Ca^{2+} flashes. A Ca^{2+} wave is defined as an increase in intracellular Ca^{2+} that propagates across a whole cell, while a Ca^{2+} flash is a temporary increase in global intracellular Ca^{2+}. The number of cells showing Ca^{2+} waves and the frequency of periodic Ca^{2+} waves was higher in cells at high density than those at low density. In contrast, neither the number of the cells showing Ca^{2+} flashes nor the frequency of periodic Ca^{2+} flashes showed significant differences between high and low cell density cultures. Although the roles of Ca^{2+} waves and flashes in SMCs are still unclear, this study indicated that the precontractile activity of cultured bovine oviductal SMCs can be evaluated using Ca^{2+} imaging methods. The regulatory mechanisms and roles of each Ca^{2+} dynamic pattern in oviductal SMCs will be investigated in future studies. This study is supported by JSPS KAKENHI (JP17H05041).

Function of the porcine oviduct under different physiological and hormonal conditions Introduction

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Oviductal epithelium is actively involved not only in transport, gametes maturation and fertilization but it's also the source of many factors occurring in oviductal fluid eg. prostaglandins, cytokines, various growth factors and others, which creates proper environment for early embryo development. New methods used in improving animal breeding, like in vitro fertilization, embryo transfer or cloning, require modification of the estrous cycle and gaining a high degree of control over the timing of ovulation as well as inducting superovulation. Oestrous synchronization and superovulation are common tools used for obtaining oocytes and embryos. However, it has been recognized that pharmacological manipulations of the oestrus cycle, apart from the expected effects, may also lead to some unwanted disturbances in longer term regulation of female reproductive tract. The most popular protocol for oestrous cycle induction involves applying a combination of equine chorionic gonadotropin (eCG) for follicular growth stimulation and human chorionic gonadotropin (hCG) to induce ovulation. Presented study was performed to determine the influence of insemination as well as treatment with hCG and eCG on expression of main factors determining oviductal function: VEGF- and prostaglandins- synthesis pathways in the porcine oviducts on Day 3 after insemination. Our study revealed that hCG /eCG treatment change prostaglandins and VEGF synthesis in dose dependent manner. Synchronization of ovulation caused decrease of expression of PTGS2 and VEGFA protein as well as decrease of PGE_2 content in the porcine oviductal isthmus, but amount of $PGF_{2\alpha}$ in oviductal tissue of gilts treated with hCG /eCG was significantly elevated. Furthermore , the PGE_2 to $PGF_{2\alpha}$ ratio in isthmus decreased significantly after hormonal treatment. Superovulation increase PGFS and CBR1 expression. Insemination itself increase expression of *PTGS2*, *mPGES* mRNA and mPGES protein and PGE2 content, but decrease VEGFA protein expression in isthmus. *Differential expression of the factors analyzed in gilts treated with exogenous gonadotrophins suggests that hCG/ eCG stimulation affects prostaglandins and VEGF synthesis pathways*

Because mechanisms responsible for this regulation remain unclear , the *in vitro* study was performed to determine the effect of hCG and FSH on cultured porcine oviductal epithelial cells (POEC). POEC, obtained in periovulatory period, were incubated with hCG (1ng/ml or 50 ng/ml), FSH (10 ng/ml) or hCG/FSH (1ng/ml or 50ng/ml) for 24 or 48 hours. Gene expression for prostaglandin and VEGF synthesis pathways were determined by qPCR and PGE2, PGFM and 6-keto PGF1α content in the incubation media using EIA kit. Statistical analyses were performed using two-way ANOVA, followed by Bonferroni's post hoc test.

POEC cultured with FSH alone showed decreased expression of PGs synthesis. Incubation of POEC with FSH alone decreased prostacyclin synthase (PGIS) levels, but without any influence on prostacyclin synthesis and IP receptor expression. We observed decreased

CBR1 mRNA expression, which coincided with PGFM concentrations in the incubation media. This strongly confirms the major contribution of the CBR1 enzyme in the synthesis of $PGF_2\alpha$ by oviductal tissue via PGE_2 conversion and the direct influence of FSH on $PGF_2\alpha$ synthesis. This study, in addition to the PGs data, resulted in altered expression of the VEGF system in POEC treated with FSH and hCG. The Flt-1 and KDR mRNA levels were lowered after incubation with 10 ng of FSH.

In conclusion, this study showed that POEC stimulation with hCG and FSH dramatically change PGs and VEGF system synthesis pathway. Any disturbances in its expression may change the local environment within the oviduct and in consequence affect gamete transport and fertilization and/or embryo early development.

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The effect of lysophosphatidic acid (LPA) on ovarian follicle function, oocyte fertilization and early embryo development in cows

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Assisted reproductive technologies (ART), including *in vitro* embryo production (IVP) have been successfully applied to animal reproduction with the aim of breeding strategies for improved production of valuable, healthy offspring. Despite the progress in IVP techniques over the years, further improvements of *in vitro* embryo culture systems are required for the enhancement of oocyte and embryo developmental competence, therefore continued effort to define the optimal media for IVP is still needed. The presented data focus on the current knowledge of lysophosphatidic acid (LPA) as a potential supplement of oocyte maturation, fertilization and embryo culture media as well as current views on the potential involvement of LPA signaling pathway during early embryo development.

Our research proved that LPA may exert regulatory influence not only on the bovine uterus, but also ovary and preimplantation embryo. In the ovary, granulosa and theca cells of the follicle as well as steroidogenic cells of the active corpus luteum are the source and target for LPA. In the bovine ovarian follicle LPA regulated follicle development via the stimulation of estradiol (E2) production. LPA also modulated TNFα dependent apoptosis of the granulosa cells in the atretic and transitional follicles. Moreover, this lysophospholipid participated in the induced by caspases, apoptosis of granulosa cells, in the atretic follicles via the activation of LPAR2 and LPAR3. On the other hand, LPA through LPAR1, also influenced granulosa cells viability, stimulating MCL1 expression and inducing anti-apoptotic processes in healthy follicles. We documented that in the healthy group of ovarian follicles, LPA acting via LPAR1 stimulated the expression of ERβ, interacted with E2 and thus influenced on granulosa cells differentiation and proliferation.

At the estrous cycle, after ovulation, during CL formation and progesterone (P4) secretion, LPA exerted auto- and para- crine role in the bovine ovary. At the time of P4 secretion, the well-known luteolytic factors such as nitric oxide (NO) or TNFα with IFNγ could not induce luteolysis in the presence of LPA. This protective, on steroidogenic cells, LPA role might occur in two ways. During functional luteolysis, LPA abrogated the inhibitory influence of NO or TNFα with IFNγ on P4 synthesis in the CL. On the other hand, LPA inhibited, dependent on cytokines and NO structural luteolysis of the CL via the influence on the proapoptotic factors in steroidogenic cells (activity of caspaze 3, Bax/Bcl2 artio, Fas/FasL complex and TNFα/TNFR1).

During the estrous cycle in the cow, LPA originating from the CL played auto/para-crine role in its development and P4 synthesis. On the other hand, during early pregnancy, LPA, being synthesized mainly in the uterus, exerted two kinds of influence on the CL. Indirectly stimulating the synthesis and secretion of endometrial PGE_2 or through the stimulation of IFNτ dependent gene expression in the CL. During the luteal steroidogenesis, we documented that LPA stimulated P4 secretion via the influence on the terminal enzyme in its synthesis - 3βHSD.

Moreover, we demonstrated that LPA augmented the stimulatory effect of interferone tau (IFNτ) on the IFNτ- dependent gene expression (OAS1 and ISG15).
We also found that oocyte and also blastocyst can be the place of LPA synthesis and target of its action in the cow. During the process of in vitro oocyte maturation, LPA increased the number of metaphase II oocytes, which resulted in the enhanced oocyte maturation rate. Moreover, we documented that the supplementation of the maturation medium with LPA decreased the number of apoptotic nuclei in the cumulus cells thus inhibited apoptosis in the oocyte-cumulus complex. We also found that the supplementation of the maturation medium with LPA increased glucose uptake via the influence on mRNA for glucose transporter (GLUT1) expression and also increased lactate production via the stimulation of mRNA for phosphofructokinase (PFKP) in the oocyte-cumulus complexes. The obtained data account for LPA–dependent redirection of glucose metabolism towards glycolysis. Moreover, LPA stimulated the expression of amfiregulin (AREG) and epiregulin (EREG) during cumulus expansion at oocyte maturation. At last, LPA increased the quality of in vitro matured oocytes via the influence on the expression of oocyte quality markers- follistatin (FST), growth and differentiation factor 9 (GDF9) in the oocyte and catepsins (CTS) B, K and Z in the cumulus cells. On the other hand the supplementation of the culture medium with LPA stimulated the expression of OCT4, SOX2, IGF2R, BAX and BCL2 in the preimplantation blastocysts.
Summarising, the obtained data enhance the existing knowledge of the bovine reproductive system function as well as the processes during early embryo development, which in the future might become the theoretical background for the optimization of assisted reproduction techniques in cows as well as help to implement new diagnostics methods of early embryo mortality.

The history and current view of polish konik horse

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Polish Konik horses are the only native breed of horses originating directly from wild tarpans (*Equus caballus gmelini* Ant.), which could be found in the areas of former Poland, Lithuania and Prussia until the end of the 18th century, and in some areas even up to the beginning of the 19th century. At that time, tarpans living in the wild were caught and then often

used to mate with local horses. In 1936, professor Tadeusz Vetulani, researcher and propagator of primitive horses, began an experiment to prove that Polish Konik horses originated from the forest variety of tarpans (*Equus caballus gmelini* Ant. form of *silvatica* Vet). For that purpose, a group of horses, most closely related in their exterior appearance to tarpans, was purchased from the local inhabitants and placed in a forest reserve in Białowieża. After professor Vetulani's death in 1952, the experiment on the restoration of forest tarpans was stopped. It was resumed in 1955 in Popielno (53°45'16.4"N, 21°37'42.1"E), where Polish Konik horses breeding has been continued up till now in two maintenance systems: the reserve and the stable ones. Polish Konik horses are an excellent model for analysing the processes and behaviour patterns of their wild ancestors, as well as in the wild horse population. Stud books for this breed have been published since 1962 by the Polish Horse Breeders' Association (PZHK). Since 1984 it has become a closed studbook and any infusion of a different breed is unacceptable. Through analysis of the numbers of Polish Koniks one will find a constant upward tendency in the population. Currently, about 1447 mares and 158 stallions are active in the breeding (2018). In accordance with the assumptions adopted in the FAO program for the preservation of genetic resources of farm animals based on the convention on biological diversity, this breed is still considered as facing extinction because the number of breeding mares in the European Union does not exceed 5000.

A sustainable and rationally conducted animal husbandry assumes maintaining biodiversity, and thus a diversified genetic pool, and the conservation of valuable phenotypic

features. Breeding selection conducted to obtain and improve different breeds of horses has largely contributed to the genetic depletion of many breeds, which has led to a decline in horse health, lack of resistance to adverse environmental conditions, vulnerability to injuries and negative factors in the environment. The widely-conducted natural selection in the case of Polish Konik horses, i.e. with a very limited human intervention, resulted in the creation of a horse breed resistant to environmental conditions. The above-average resistance and endurance of the Polish Konik horses is especially visible in animals living in a forest reserve. Of course, under reserve breeding conditions, the cultivation is generally limited to catching foals, so as to maintain a relatively constant number of horses in a particular area. On the other hand, rigorous selection should be conducted in stable breeding. However, one of the assumptions of the breeding program for Polish Konik horses is the increase in population. In recent years it has been increasing steadily, but considering the fact that the work on reproducing the breed started with very limited breeding material, the changes in that breed should be constantly monitored. The analysis of the affiliation of stallions and mares to separate genealogical lines indicates their uneven representation [Jaworski, 1997], which influences the insufficient diversity of genetic variability and even threatens its loss. Pedigree analyses show that in the Polish Konik horses population, in which 35 female families and 6 male lines were identified, only some present satisfactory breeding activity. Among the male lines, the stallion lines of the lowest representation include the Liliput and Glejt I lines. Among 35 female families, only 16 are active and 6 of them present clear increases (Liliputka I, Karolka, Zaza, Urszulka, Tarpanka I, Traszka), while the remaining ones show either slight development or stagnation (Tygryska, Popielica, Wola, Białka, Ponętna, Misia II, Dzina I, Tunguska, Bona, Geneza). The breeding is aimed at strengthening a variety of genetic characteristics, striving for a healthy offspring, and improving animal functional traits. If the selection were indeed carried out in accordance with the above objectives, it would exclude from reproduction some individuals which represent rare genealogical lines, but are less likely to reproduce and are less likely to get offspring. The unique nature of the breed and the limited amount of breeding material necessitate a strict reproductive control in Polish Konik horses. The mating importance in this breed is related to the possibility of using a much larger number of stallions to reproduce, which at the same time slows the growth of inbreeding [Górecka and Jezierski, 1995] than in the case of artificial insemination with the material from a small group of males. It should be noted that the Polish Konik horse population is limited and the stud books for this breed are closed, which means that no horse from outside the breed can be introduced to breeding. Assuming that there is also a reserve breeding of Polish Konik horses, where the herds are usually created in a spontaneous manner, a steady increase in inbreeding connected with this breed may be expected, leading to deterioration in the health of the breed, manifested, for example, in reproductive problems. Nevertheless, spontaneous ethological mechanisms, including among others expulsion of maturing daughters from the herds belonging to the stallions or excluding the mares from mating by their fathers, cause the growth of inbreeding to be lower than expected. Moreover, by analysing inbreeding coefficients, Jaworski and Jezierski [1999] concluded that it amounts to 0.237 between the stallions and mares within the herds and 0.33 between the stallions and mares from outside the herds. Thus, this mechanism effectively protects the population from the increase in homozygosity.

The utility value of Polish Konik horses both in stable and outdoor keeping is assessed on the basis of field tests. Mares and stallions have to pass an initial performance test before they may be entered in the Studbook. Then, at the age of 3-4 years stallions are to undergo an obligatory basic performance test under saddle or in harness and mares have 4 years to pass the test starting from the day of their registration. These tests serve to improve gaits and the traits important in recreational applications. Movement of Koniks has improved much in recent years both in terms of the length of stride and engagement of the hindquarters. In terms of conformation Polish Konik horses are more similar to draft horse breeds, while their other advantages such as their gentle nature, friendly attitude to a human and willingness to work, contribute to the fact that they are mostly used for riding both by children and adults. Thanks to a small size, the appropriate temperament and ease of making contact with humans they are popular in hipotherapy. A unique form of the usage of Polish Konik horses is their role in protection of the landscape, such as the prevention of plant succession on open grasslands. Biting off and trampling stems of young plants, and gnawing the bark from young trees slows down growth of undesired plants and reduces their succession. By acting as so-called "living lawn mowers" Koniks help to control the vegetation and carry out landscape management.

The forest reserve in Popielno is located on a peninsula, surrounded by four lakes. Since 1962, the total area of the forest available to freely roaming herds of the Polish Konik horses has been 1620 ha. Interference by humans in the life of the herds is restricted to a minimum. In a majority of cases the herds had formed naturally, through fights for mares or when a new stallion took control over the herd after the removal of the old one. The composition of the herds is stabile and usually has not changed for years, with exception of the young mares, which are driven away from the home herds by their fathers. This spontaneous ethological mechanism, which leads to migration of the young mares between herds, safeguards against a drastic increase in inbreeding. An excellent adaptation of the Polish Primitive Horses to the difficult environmental conditions is proved by their high fertility. The incidence of pregnancies reaches 91% on average over the whole 40 years period studied. The lifespan of mares in the forest reserve is about 30 years and they remain fertile until the age of 24-27 years. The cases of retained fetal membranes or dystocia are sporadic in this breed. The cases of twin pregnancies naturally terminated were also sporadic; however once, in 1989, a twin pregnancy resulted in fully developed twins. The length of pregnancy may differs in the Polish Konik mares kept in stable. The shortest observed pregnancy was 320 days and the longest was 358 days with an average 331,06±8,2 days. An average pregnancy length in case of dropping a colt was 332,46±7,17, and in case of dropping a filly was 330,5±6,2 days. The breeding carrier of the Polish Konik mares is usually very intensive and expanded for years. The satisfying reproductive rates are the evidence of a high reproductive potential of Polish Konik horses. Breeding the Polish Konik horses in Popielno has saved extinction of a unique population originating from the ancient tarpan. At present, the population of the Polish konik is increasing, however, it must be noted that restoration of the breed began with very limited breeding material and therefore special precautions should be taken to conserve the genetic pool and all changes occurring within the population must be monitored carefully.

Status and prospect of Horse breeding and reproductive treatment in Japan

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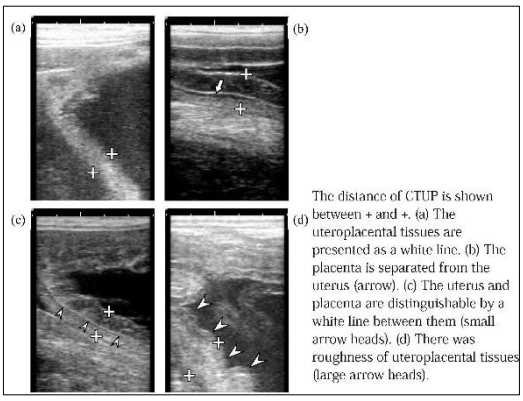
Horse industry in Japan

Although there are only 80,000 horses in Japan, 55% of them are Thoroughbreds (TB) for the racing industry and others include heavy draft horses, riding horses, and native ponies. Thoroughbred breeding in Japan stands fifth in the world. Approximately 7000 TB foals are born every year in Japan. And about 95% of their broodmares are bred and raised in Hokkaido, a north island. None of the other regions of the world where Thoroughbreds are bred and raised have severe cold climate in the winter as it has in Hokkaido (see photo). For several years, Japan Racing Association (JRA) has also been conducting multifaceted research related to racehorses, such as that related to nutrition, exercise physiology, ethology, genetic science, and clinical sciences and pathobiology. One area of advanced research that has recently attracted considerable attention is equine reproduction for efficient breeding.

Obihiro is famous for holding heavy draft horse racing (see photo). Approximately 60% of these horses are bred in the eastern part of Hokkaido. Thus, our university should focus on the problem in Heavy draft horses in addition to other breeds.

Diagnosis of placental disorder in Heavy draft mares

There are a lot of pregnancy loss caused by infectious or non-infectious cases in Heavy draft horses. In this study, combined thickness of uterus and placenta (CTUP) and uteroplacental imaging were investigated in heavy draft horses. In 35 pregnant heavy draft horses, CTUP was measured and ultrasonographic images were obtained in Month 7–12 of pregnancy. Mares were divided into three groups: those pathologically diagnosed as having placentitis (placentitis group, n = 3); those who had abortion, premature birth, or fetal malformation (abnormal group, n = 7); and the remaining 25 animals who had no abnormal findings (normal group). In the normal group, CTUP increased as pregnancy progress and was higher than those



The distance of CTUP is shown between + and +. (a) The uteroplacental tissues are presented as a white line. (b) The placenta is separated from the uterus (arrow). (c) The uterus and placenta are distinguishable by a white line between them (small arrow heads). (d) There was roughness of uteroplacental tissues (large arrow heads).

reported previously in Thoroughbreds. Increased CTUP was considered to reflect placentitis and abnormal pregnancies. Ultrasonographic images showing placental separation were obtained in 67% of the placentitis group (2/3), 29% of the abnormal group (2/7), and 20% of the normal group (5/25). Ultrasonographic images showing uteroplacental roughness or distinguishability, and pathological placental edema were observed even in the normal group (figure). These findings suggest that the detection of increased CTUP and placental separation may become a diagnostic aid for detecting abnormal pregnancy in heavy draft horses [1].

Embryos transfer from a Hokkaido native pony after artificial insemination using frozen semen

Embryo transfer (ET) technology allows the donor mare to potentially produce multiple foals in a year. In Japan, there has been no report of foal born through ET after artificial insemination using frozen semen. In this season, special riding crossbred horses (Connemara pony x Hokkaido native pony) were produced through ET technology. A non-surgical transcervical procedure was used to collect embryo from the uterus of the donor mare at day 7 post-ovulation and transferred fresh into the uterus of recipient mare. Four embryos were collected from a single donor mare and were transferred to recipients in spring 2018 [2]. Three out of 4 recipient mares (75 %) established successful pregnancy and delivered a healthy foal each in 2019 (see photo). These are the first foals produced through embryo transfer in Japan after artificial insemination using frozen semen. We are expecting that this new crossbred would be an ideal riding breed for disable people.

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