Japan Society for the Promotion of Science
&
Polish Academy of Sciences

POLISH-JAPANESE JOINT SEMINAR CUTTING-EDGE REPRODUCTIVE PHYSIOLOGY –A PATH TO PREGNANCY –

26 September – 01 October, 2015
Gdańsk, Poland

Welcome to the Conference

Dear Colleagues,

We are pleased to welcome you in Gdańsk for the "International Conference on Biology and Pathology of Reproduction in Domestic Animals".

The Conference is especially intended to encourage interaction between researchers, university lecturers and Vet practitioners. Therefore, this year Conference is consisted of three parts:

1. Second Meeting on "ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS" and two satellite meetings**:**
2. Satellite meeting I: "CUTTING-EDGE REPRODUCTIVE PHYSIOLOGY – A PATH TO PREGNANCY" (joint Polish-Japanese Seminar under agreement of Polish Academy of Science and the Japanese Society for promotion of Sciences),
3. Satellite meeting II: "EQUINE REPRODUCTION IN A PILL - LECTURES FOR PRACTICIONERS".

The aim of the Conference is to provide current knowledge about the ovarian and uterine biology and morphology, as well etiology and pathogenesis of endometritis, clinical and subclinical endometritis, new diagnostic methods and new treatment strategies. Moreover, the impact of endometritis on reproductive health and animal productivity will be also discussed.

We want to thank all of our invited speakers that have been accepted our invitation to come to Olsztyn. We are very pleased to have them with us in our meeting and look forward to hearing about the interesting studies that they do.

Finally we want to thank all of participants. We are grateful to you for your support and discussion. We wish all of you many fruitful discussion, meeting old friends, making new partnerships and having a pleasant stay in Gdańsk.

Local organizing and scientific committee

Dariusz J. Skarżyński, Marta Siemieniuch, Karolina Łukasik, Anna Szóstek, Antonio M. Galvao (Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research Polish Academy of Science in Olsztyn, Poland)

Tomasz Janowski, Anna Rapacz-Leonard, Wojciech Barański (Department of Animal Reprodcution, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland)

Roland Kozdrowski (Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Science, Poland)

GREETING

Kiyoshi OKUDA

Laboratory of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, 700-8530 Okayama, Japan; E-mail: kokuda@okayama-u.ac.jp

I am very pleased to be in Gdansk as an organizer of Japanese side for our Poland and Japan joint seminar. The present seminar is the third meeting with Polish and Japanese scientists. The first seminar was held in Krakow 2005, and the second was in Iwate in Japan 2011 with the same big title "Cutting-edge Reproductive Physiology". The purpose of this seminar is to exchange the current knowledge of reproductive physiology as well as technology. Both previous seminars were very fruitful.

I wish that the present joint seminar will also produce good results for the attendants of both countries, and that new collaborative projects between both countries will be duscussed and established during the meeting.

Polish-Japanese Joint Seminar Cutting-edge Reproductive Physiology – a path to pregnancy

September 28-30, 2015

Stanislaw Moniuszko Academy of Music in Gdansk, Lakowa street 1-2,

Gdańsk, Poland

September 28 th, Monday

Plenary session I

Chairs: Dariusz J. SKARŻYŃSKI and Kiyoshi OKUDA

9.00 - 9.15 **Opening Remark & Review**: Kiyoshi OKUDA *(Lab. of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, Japan)*

9.15 - 10.00 **Plenary lecture I: Kei-Ichiro MAEDA: Brain mechanism controlling mammalian reproduction: An overview** *(Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan)*

10.00 - 10.45 **Plenary lecture II - Adam ZIĘCIK: Early pregnancy recognition - lessons learned from genes expression studies** *(Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland)*

10.45 - 11.15 Coffee break

Gamete and embryo development

Chairs: Koji KIMURA and Izabela WOCŁAWEK-POTOCKA

11.15 - 11.35 Ken SAWAI, *Iwate, Japan*: Molecular mechanisms involved in segregation of inner cell mass and trophectoderm lineages in bovine and porcine embryos

11.35 - 11.55 Zofia MADEJA, *Poznań, Poland*: The effect of Wnt/β-catenin signalling on bovine preimplantation development – prospects for bovine ESC derivation

11.55 - 12.10 Paweł KORDOWITZKI, *Neustadt am Ruebenberge, Germany*: Effects of resveratrol supplementation during in vitro maturation and in vitro fertilization on developmental competence of bovine oocytes

12.10 - 12.25 Nobuyuki SAKURAI, *Iwate, Japan*: Effects of downregulating OCT-4 and CDX2 transcripts on early development and gene expression in bovine embryos

Gamete transportation and oviduct functions

12.25 - 12.45 Yuki YAMAMOTO, *Okayama, Japan*: How is oviductal motility controlled? Production mechanisms of local factors which regulate smooth muscle contraction and relaxation in cattle

12.45 - 13.05 Anna DUSZEWSKA, *Warszawa, Poland*: Functional morphology of cattle oviduct: comparison of morphology of bovine epithelial cells (BOECs) at an elevated temperature

13.05 - 13.20 Yoshihiko KOBAYASHI, *Okayama, Japan*: Region-specific roles of endothelins in the bovine oviduct: Regulation of nitric oxide synthesis and spontaneous waves of contraction and relaxation

13.30 - 14.30 Lunch

Maintenance of pregnancy: role of corpus luteum and uterus

Chairs: Adam ZIĘCIK and Naoko INOUE

14.30 - 14.50 Ryosuke SAKUMOTO, *Tsukuba, Japan*: Changes in the gene expression profiles of bovine corpus luteum during early pregnancy

14.50 - 15.05 Kaya WATANABE, *Obihiro, Japan*: Role of Bmal1 clock gene on corpus luteum formation of pregnancy in mice ovary

15.05 - 15.20 Kazuhisa HASHIBA, *Okayama, Japan*: An increase in the level of α2,6-sialic acid inhibits galectin-1 binding to glycan during luteolysis

Mechanisms of maternal recognition of pregnancy and implantation failure

Chairs: Marta SIEMIENIUCH and Ken SAWAI

15.20 - 15.40 Koji KIMURA, *Okayama, Japan*: Evaluation of an alternative embryo transfer strategy to mitigate early embryonic loss and differential gene expression in endometria of fertility and sub-fertile cattle

15.40 - 16.00 Izabela WOCŁAWEK-POTOCKA, *Olsztyn, Poland*: The effect of lisophosphatidic acid (LPA) on the embryo-maternal cross-talk in cows

16.00 - 16.20 Katarzyna BUSKA-PISAREK, *Wrocław, Poland*: Early embryo-maternal communication in natural pregnancy and after embryo-transfer in mice

16.20 - 16.35 Ken GO HAYASHI, *Tsukuba, Japan*: Temporal expression of vascular endotherial growth factor family members in the bovine endometrium during peri-implantation period

16.35 - 17.00 Coffee break

Biology and pathology of placenta

Chairs: Takashi SHIMIZU and Tomasz JANOWSKI

17.00 - 17.20 Marta SIEMIENIUCH, *Olsztyn, Poland*: Ovarian and placental molecular mechanisms responsible for pregnancy maintenance and prepartal luteolysis in cats

17.20 - 17.40 Anna RAPACZ-LEONARD, *Olsztyn, Poland*: Equine fetus does not 'hide' from the mother's immune system during pregnancy, parturition and fetal membranes retention

17.40 - 17.55 Joanna JAWORSKA, *Olsztyn, Poland*: Does equine fetus express only maternally inherited Major Histocompatibility Complex I (MHC I) in order to protect long lasting pregnancy?

20.00 - 22.00 Welcome reception

September 29 th, Tuesday

Central regulations of reproductive functions

Chairs: Kei-Ichiro MAEDA and Anna DUSZEWSKA

10.10 - 10.30 Naoko INOUE, *Nagoya, Japan*: Brain mechanism underlying ovulation in mammals

10.30 - 10.45 Yuta SUETOMI, *Nagoya, Japan*: Molecular Cloning and Identification of the Transcriptional Regulatory Domain of the Goat Neurokinin B Gene TAC3

10.45 - 11.00 Satoshi OHKURA, *Nagoya, Japan*: Electrophysiological Technique for Monitoring the Hypothalamic Mechanism Regulating Pulsatile GnRH Release in Goats

11.00 - 11.15 Sho NAKAMURA, *Tokyo, Japan*: Neonatal kisspeptin is required for defeminization of the brain mechanism controlling sexual behaviors in male rats

11.15 - 11.45 Coffee break/Poster session

11.45 - 12.00 Youki WATANABE, *Nagoya, Japan*: Involvement of preoptic kisspeptin neurons in estrogen positive feedback to induce luteinizing hormone surge in both female and male Japanese monkey

12.00 - 12.15 Kana IKEGAMI, *Nagoya, Japan*: Involvement of cell-to-cell communication via gap junctions in NKB-NK3R signaling-induced synchronous discharges of KNDy neurons

Ovarian functions (Follicular growth, Follicular functions, Ovulation mechanisms, Luteolysis)

Chairs: Ryosuke SAKUMOTO and Dariusz SKARŻYŃSKI

12.15 - 12.35 Takashi SHIMIZU, *Obihiro, Japan*: Effect of endotoxin on ovarian follicle function in domestic animals

12.35 - 12.55 Anna ZIELAK-STECIWKO, *Wrocław, Poland*: Genomic portrait of ovarian follicle growth regulation in cattle

12.55 - 13.15 Maciej MURAWSKI, *Kraków, Poland*: Ovarian and endocrine function after hormonal induction of ovulation in seasonally anovular goats

13.15 - 13.30 Antonio GALVAO, *Olsztyn, Poland*: Nodal promotes vascular regression via Thrombospondin-1 pathway during luteolysis in the mare

13.30 - 13.35 Closing remark & Review: Dariusz J. SKARŻYŃSKI *(Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland)*

13.00 - 14.30 Lunch

20.30 - 23.00 Gala Dinner

September 29 th, Wednesday

12.55 - 13.00 Closing Ceremony

13.00 - 14.00 Lunch

Speakers

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 immuno-endocrine, 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) Pathogenesis of *endometritis* both, in mares and cows, and *endometrial fibrosis* in mares. He carried out research as visiting fellow/professor at: 1) Okayama University, Japan (postdoctoral fellow of the JSPS; 1997-1999, 2 years); 2) Graduate School of Natural Science and Technology, Okayama University, Japan (visiting professor, 2003-2013, in total 16 months); 3) Faculty of Veterinary Medicine, University in Lisbon, Portugal (visiting professor, 2008-2014, in total 12 months); 4) Interdepartmental Centre for Studies on Biology of Reproduction, University of Siena, Siena, Italy (visiting professor, 2012, 2013); 5) Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, Israel (visiting professor, 2013).

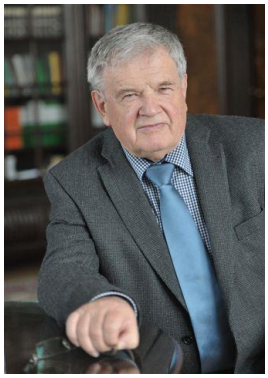
Kiyoshi OKUDA, Japanese Organizer of the Joint Seminar

Kiyoshi OKUDA, DVM, Dr. med. vet., Ph.D., is a Professor of Reproductive Physiology at Okayama University, Okayama, Japan. His research focuses on mechanisms in regulating bovine corpus luteum function. His team has been studying local regulation of luteolysis including molecular mechanisms of luteal cell death and uterine PGF2α secretion in cattle. They found that a variety of local factors including cytokines as TNFα play big roles not only in regulating luteolysis but also in uterine PG synthesis. They has also demonstrated that a hypoxic condition, which is induced by bleeding at ovualtion 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, 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.

Kei-ichiro MAEDA

Kei-ichiro MAEDA, Ph.D., is the Professor of Theriogenology of the University of Tokyo, Japan, who has been working on the neuroendocrinology of reproduction, especially the mechanism regulating gonadotropine-releasing hormone (GnRH) release in various animals. He graduated the Department of Veterinary Medicine, the University of Tokyo in 1980 to get the degree of DVM. He did his PhD in the University of Tokyo for the research on the seasonal reproduction in goats. He then moved to Nagoya University to work on the mechanism generating GnRH surges and pulses for more than 27 years before getting the professorship in the University of Tokyo. He is now chairing the Department of Veterinary Medicine of the University of Tokyo and the President of the Society for Reproduction and Development. One of his major contributions in reproductive biology is to clarify the role of kisspeptin in generating surges and pulses of GnRH. The research is still going on in collaboration with the Nagoya University research group led by Prof. Hiroko Tsukamura by generating various kinds of genetically-modified rodents. Prof. Maeda is also trying to apply kisspeptin research to the development of oral drugs controlling gonadal activities in wild and domestic animals. The other research project he is promoting now is to clarify how nutrition regulates the activity of hypotahalamo-pituitary-gonadal (HPG) axis. His research in this area is now focusing on the energy sensing mechanism in the brain. Ependymocytes lining the cerebroventricular wall might be capable of sensing energy substrates to control reproduction as well as feeding.

Adam J. ZIĘCIK



Adam ZIĘCIK, Ph.D., received PhD (1974) and DSci (1983) degrees in animal physiology at University of Agriculture and Technology in Olsztyn. He carried out research as visiting fellow/professor at Babraham (UK), NDSU and NCSU (USA) in 1979/1980 and 1984-1989 and Universities of Turku and Oulu (Finland), ITT Mariensee (Germany), URE Okayama University (Japan) and INIA Madrid (Spain). Prior assuming his present responsibilities he worked as Lecturer(1974-1984) and Senior Lecturer(1983-1984), Institute of Animal Physiology and Professor (1988-89) UA-T Olsztyn; Head of Department and Professor (1989-2000), Scientific Director (1992-1995) and Director (2000-2012) Institute of Animal Reproduction and Food Research of Pol. Acad. Sci. (PAS) in Olsztyn, Vice-President of PAS (2012-2014). He has been Editorial Board Member of several scientific journals (Reprod Dom Anim, Dom Anim Endocrinol, Reprod Biol, Anim Sci Pap Rep), Member Board of ESDAR (1997-2002), Chairman of Society of Biology of Reproduction in Poland (1999-2005). He was also member of ICAR Standing Committee (1992-2008) and ICPR (2005-2013). His research interests include basic reproductive endocrinology of domestic animals (mainly pigs), especially functions of gonadotropins, endometrium, corpus luteum and embryo signals.

Ken SAWAI

Ken SAWAI, Ph.D., is a Professor of Department of Animal Science, Faculty of Agriculture, Iwate University. He is interested in the epigenetic regulation system in early embryos of domestic animals such as cattle and pig. His research focuses on 1) mechanisms in regulating the gene expression of bovine embryos derived by somatic cell nuclear transfer (SCNT) and 2) segregation and function of inner cell mass (ICM) and trophectoderm (TE) lineage during pre-implantation and post-implantation development of bovine and porcine embryos. Dr. Sawai and his collaborators found aberrant gene expression patterns, DNA methylation and histone modification in bovine SCNT embryos. They have reported also those epigenetic statuses in bovine SCNT embryos are corrected as a result of demethylation and retention of methylation as the embryo differentiates and TSA treatment of SCNT embryos. Furthermore, they have reported the role of OCT-4 or CDX2 for segregation and functionalization of ICM and TE lineages in bovine and porcine embryos. Dr. Sawai also focuses on improvement of *in vitro* culture system using by evaluation of gene expression status in bovine and porcine IVF embryos.

Zofia MADEJA

Zofia MADEJA, Ph.D., Eng. is an Assistant Professor at the Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poland. Her main field of interest is focused on developmental biology and stem cell research. Her PhD thesis was dedicated to leptin and leptin receptor gene and protein expression in bovine pre-implantation embryos, for the first time indicating a polar distribution of these factors within oocytes and embryos of this species (Madeja et al., 2008, Animal). Shortly after defending her PhD thesis, she moved to Cambridge, UK where she became involved in the study of early embryonic cell polarity and cell fate in the early mouse embryo (Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK). From September 2006 until April 2009, Dr Madeja worked as a PostDoc in Dr Myriam Hemberger's group (The Babraham Institute, Cambridge, UK) on a project that was aimed at elucidating the expression of paternal antigens on the surface of mouse trophoblast and their impact on reproductive outcome. Dr Madeja was also involved in a research on the epigenetic restriction of embryonic cell fate. In September 2009 she obtained a fellowship from the Foundation for Polish Science and returned to her PhD department. Since 2010 she has directed a research grant dedicated to the study of „Pluripotency markers expression and genomic stability of bovine embryonic stem cells" and in 2011 she obtained a Fellowship from the Polish Minister of Science and Higher Education for "Outstanding Young Scientists". Her current work investigates this problem and focuses on searching for the optimal conditions to derive stable ESC lines from cattle.

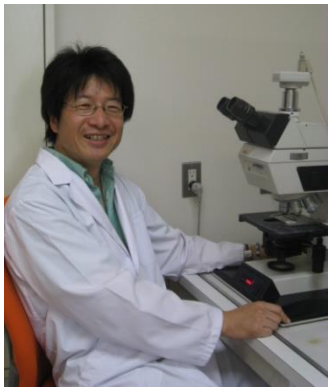
Yuki YAMAMOTO

Yuki YAMAMOTO, Ph.D., is an Assistant Professor of Graduate School of Environmental and Life Science at Okayama University, Okayama, Japan. Her research topic is regulatory mechanisms of oviductal function in cow
Oviduct provides the optimal environment for fertilization, early embryonic development and transport of gametes and embryo. Recently, our research group has newly established the isolation and culture methods of bovine oviductal epithelial and stromal cells. Using these methods, we investigate the cellular function including 1) Regulatory mechanism of transport of gametes and embryo and 2) mechanism of environmental control for fertilization and early embryonic development in the oviduct. The objective of our research is to contribute to increasing the conception rate of mammals such as domestic animals and human.

Anna Maria DUSZEWSKA

Anna Maria DUSZEWSKA, Ph.D., is an Associate Professor in the Division of Histology and Embryology, Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences. She has received her PhD in Veterinary Medicine (1995) from Warsaw University of Life Sciences. In 1996, she joined the Institute of Genetics and Animal Breeding, Polish Academy of Sciences as an Assistant Professor in the Experimental Embryology Department, where she received habilitation in 2006. From 2006 to 2008 she worked as an Assistance Professor in that Institute. Since 2009, she has been working as an Associate Professor in the Division of Histology and Embryology, Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences. She has been engaged in lecturing in Embryology, Histology, Cell biology and Genetics. She has been the supervisor for 8 Masters and 3 PhD students. She has been interested in research including *in vitro* production of cattle embryos and creation of transgenic cattle. Currently, she focuses on the study of the influence of elevated temperature on the oviduct and early cattle embryo development in the aspect of maternal-embryo interaction. She has been engaged in *in vitro* production of wisent embryos. Anna Maria Duszewska has realized polish and international projects (NCS, COST) and published her research in, amongst others, PLOs One, Journal of Physiology and Pharmacology, Theriogenology, Reproduction Fertility and Development. She is an expert for the Federation of Veterinarians of Europe (FVE) and National Program FORESIGHT 2020 and a member of the Epiconcept Action FA1201 (COST), Polish Society of Reproductive Biology and others.

Ryosuke SAKUMOTO



Ryosuke SAKUMOTO, Ph.D., is a Principal Researcher of Animal Physiology Research Unit, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Japan. His research focuses on local regulation of corpus luteum (CL) and uterine function during estrous cycle and pregnancy in cows. He and his collaborators found that the gene and protein expression profiles including cytokines/chemokines in the bovine CL and uterus have been drastically changed from the estrous cycle to pregnancy. In March 2000, he had a Ph.D degree from Okayama University under the supervision of Prof. Kiyoshi Okuda. From July 2006 until August 2008, he worked as a Research Fellow of Alexander von Humboldt Foundation at the University of Munich, Germany under the supervision of Prof. Fred Sinowatz. Dr. Sakumoto also worked as a Research Coordinator of Agriculture, Forestry and Fisheries Research Council Secretariat, Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan from April 2010 until March 2012. Currently, he works to investigate interaction between CL, uterus and conceptus at the time of maternal recognition in cows as Head of a research program on Innovative Technology for Animal Reproduction from the MAFF.

Koji KIMURA

Koji KIMURA, Ph.D., is an Associate Professor of Graduate School of Environmental and Life Science at Okayama University, Okayama, Japan. His PhD and postdoctoral studies investigated sexual dimorphism in bovine embryo development. This research was fundamental in identifying that high glucose concentration in the culture media skews embryonic sex ratio towards males. Moreover, that female embryos secrete twice the concentration of IFN-tau (the maternal recognition signal) compared with males and that this is directly associated to X-chromosome dosage and activation; a phenomenon that he investigated in both in vitro and in vivo studies. His other major research area has been the development of a novel single FSH administration method for superovulation in cattle, which has substantial commercial application. 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.

Izabela WOCŁAWEK-POTOCKA

Izabela WOCŁAWEK-POTOCKA, Ph.D., is an Associate Professor at the Department of Reproductive Immunology and Pathology, Polish Academy of Sciences in Olsztyn, Poland. Her research focuses mainly on two scientific subjects: the influence of phytoestrogens on the functions of the reproductive organs in the cow and the influence of the immune system modulators (prostaglandins and lysophosphatidic acid) on the mechanisms controlling estrous cycle and early pregnancy (especially oocyte maturation, ovulation and embryo-maternal interactions) in the cow.

Katarzyna BUSKA- PISAREK

Katarzyna BUSKA-PISAREK is a graduate of Wrocław University of Environmental and Life Sciences, faculty: microbial biotechnology. She also conducted postgraduate curse of Quality management systems and food safety and became a member of Quality Management Academic Club. Since 2011 she is a PhD Candidate at Ludwik Hirszfeld Institute of Immunology and Experimental Therapy PAS, Wrocław. Her main scientific interest is reproductive biology and immunology. Member of Polish Society for Biology of Reproduction. Currently working on grant project "Early embryo-maternal communication in preimplantation pregnancy in mice". Study is focused on investigation of short and long-rage signals during embryo-maternal dialogue. Short signals are represented by the local actions in the uterus in response to embryo appearance, long-range signals can be understood as differential activity of immune cells during pregnancy, not only in the uterus, but also on periphery. Results of splenic T CD 4+ lymphocytes proteomic analysis was published in paper "Proteome of spleen CD4 lymphocytes in mouse preimplantation pregnancy" (Chelmonska-Soyta *et al.* 2014).

Marta SIEMIENIUCH

Marta SIEMIENIUCH, Ph.D., is an Associate Professor in the Department of Reproductive Immunology and Pathology in the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn. She graduated Veterinary Medicine at the Agricultural University (currently the University of Environmental and Life Sciences) in Wrocław, Poland in 2003. Next she started PhD studies at the Faculty of Veterinary Medicine in Wroclaw and obtained PhD degree in 2006. Since 2006 she is employed in the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn in the Department of Reproductive Immunology and Pathology. Main areas of her interests are: (i) involvement of the molecular mechanisms of the feline and equine placenta in the endocrine balance; (ii) regulation of prostaglandin and steroid biosynthesis in the corpus luteum of dogs and cats; (iii) new strategies of non-surgical contraception in *Carnivora* and endocrine disturbances as a result of pharmacological contraception treatments; (iv) subclinical and clinical *endometritis* as a main problem in the breeding management in horses. She did several internships as a visiting researcher in Portugal (University in Lisbon); Switzerland (Zurich, Vet Swiss Faculty); US (Woods Hole, MA, "Frontiers in Reproduction"). She spent also 12 month internship in Okayama, Japan as a post doc under the academic supervision of Prof. Kiyoshi Okuda. Marta Siemieniuch has been a part of organizing committees for both conferences on ‘ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS’

Anna RAPACZ-LEONARD

Anna RAPACZ-LEONARD, Ph.D., is an Assistant Professor in the Department of Animal Reproduction with Clinic, at the Faculty of Veterinary Medicine, at the University of Warmia and Mazury in Olsztyn (Poland). She graduated veterinary medicine at the University of Warmia and Mazury, Olsztyn, Poland in 2007. She obtained her PhD degree in 2011, defending her thesis entitled “Selected aspects of the etiopathogenesis of retained placenta and its impact on fertility in mares”. She specializes in animal reproduction and equine diseases. Anna Rapacz-Leonard has been investigating the mechanism underlying fetal membranes retention in heavy draft mares in the area served by the clinic (province of Warmia and Mazury). She received two national grants funding her research. From her research, she published 7 original articles and 4 reviews (total IF: 12.227). Anna Rapacz-Leonard has been a part of organizing committees for both conferences on ‘ENDOMETRITIS AS A CAUSE OF INFERTILITY IN DOMESTIC ANIMALS’.

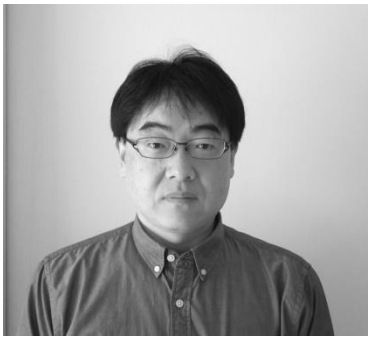
Naoko INOUE

Naoko INOUE, Ph.D., is a Lecturer of Laboratory of Reproductive Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan. Her research focuses on neuroendocrine mechanism controlling the reproductive function in mammals. Currently, she is studying neural mechanism regulating a reflex ovulation. Dr. Inoue and her colleagues have demonstrated that the mating stimulus activates kisspeptin neurons to induce ovulation *via* gonadotropin-releasing hormone (GnRH) release in musk shrews, a reflex ovulator. She is trying to generate transgenic musk shrews as a useful experimental model of the reflex ovulator and involved in the musk shrew genome project. Dr. Inoue also focuses on the mechanism controlling follicular development and degeneration. During her graduate school training at Kyoto University under the supervision of Dr. Noboru Manabe, she and her collaborators showed that death ligand-death receptor system regulates granulosa cell apoptosis in porcine ovaries.

Satoshi OHKURA

Satoshi OHKURA, Ph.D., is a Professor at the Laboratory of Animal Production Science, Nagoya University, Nagoya, Japan. He has got his Ph.D. in Agricultural Science under supervision of Professor Kei-Ichiro Maeda and late Professor Akira Yokoyama in Nagoya University in 1993. His current research focuses on the brain mechanism underlying the regulation of gonadotropin-releasing hormone (GnRH) release in ruminant species. His collaboration study with Drs. Hiroaki Okamura and Yoshihiro Wakabayashi, National Institute of Agrobiological Sciences, Tsukuba, Japan, showed, using electrophysiological technique in Japanese native miniature goats, that the kisspeptin/neurokinin B/dynorphin A (KNDy) neurons in the hypothalamic arcuate nucleus would be the intrinsic source of the GnRH pulse generator, the hypothalamic "pacemaker" mechanism that generates pulsatile GnRH secretion. He and his colleagues are trying to unravel the neuroendocrine mechanism of GnRH pulse generator to apply such basic knowledge to the improvement of conception rate and the efficiency of production in livestock.

Takashi SHIMIZU



Takashi SHIMIZU, Ph.D. is an Associate Professor of a Graduate School of Animal and Food Hygiene, Division of Animal Medical Science, Obihiro University of Agriculture and Veterinary Medicine. He is interested in the effect of endotoxin (lipopolysaccharide, LPS) on follicular cell function and development competence of oocytes during follicular development in domestic animal.

He has reported the presence of LPS in follicular fluid of large follicles isolated from uterine inflammation cows. Moreover, he found that in follicles with a high level of LPS, the concentration of estradiol was lower and that of progesterone was higher when compared to those with a low level of LPS. In addition, he observed the drastic changes in transcription levels of steroidogenesis-related genes. Using in vitro culture system for follicular cells, he found that LPS disturbed cell-specific steroid production in granulosa cells and theca cells of follicles at different developmental stages in bovine ovaries. Now, he will plan to study LPS effect of oocyte function in cattle.

Anna E. ZIELAK-STECIWKO

Anna ZIELAK-STECIWKO, Ph.D., is an assistant professor in the Institute of Animal Breeding at Wrocław University of Environmental and Life Sciences. Her interests lie in understanding the molecular regulation of ovarian follicle development. Dr Zielak-Steciwko received her M.Sc. in Animal Science at Agriculture University of Wrocław, Poland in 2002. Then she joined the Research Team led by Prof. Alexander Evans and started her PhD studies at University College Dublin (School of Agriculture, Food Science and Veterinary Medicine), Ireland. In 2007, she received her PhD in molecular biology of reproduction with an emphasis on specific groups of genes at specific stages of dominant and subordinate ovarian follicle development in cattle. Upon graduation she has taken up an academic position at Wroclaw University of Environmental and Life Sciences. Her current research interest focuses on elucidating the function of microRNAs in regulation of cattle ovarian follicle development by defining their role in follicle fate and identifying potential pathways and putative targets with use of bioinformatics tools. Her studies aim at identification of microRNAs that could induce down-regulation of expression of a number of genes that are involved in programmed cell death in the dominant follicle, as well as the genes that are decisive for the proliferate status of the cell in subordinate follicles.

Maciej MURAWSKI

Maciej MURAWSKI, Ph.D., Eng. is an Assistant Professor at the Department of Animal Biotechnology, University of Agriculture in Kraków, Poland. He is interested in the small ruminants reproduction biotechnology and physiology. His research focuses on dynamic of ovarian follicles growth in wave like pattern and possibility of its modification by use of exogenous hormones in sheep and goats. He is also involved in the studies on assisted reproductive techniques specially insemination and embryo transfer in small ruminants. Recently, with collaboration team he found that nanowater used in diluent for ram semen cryopreservation significantly improves its quality and increase fecundity of inseminated ewes (Murawski et al. Exp. Biol. Med. 2015).

Brain mechanism controlling mammalian reproduction: An overview

K.I. MAEDA and H. TSUKAMURA

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Date back a number of decades, Geoffrey Harris, a British anatomist, predicted the presence of hypothalamic hormones controlling anterior pituitary hormone release. He did a lot of excellent experiments in rats to provide the evidence indicating substances released from the hypothalamus reach the anterior pituitary through local blood circulation to stimulate or inhibit the pituitary hormone secretion which eventually regulates the functions of peripheral organs. He is the first to predict the presence of hypothalamic hormones, and therefore, is now called the Father of Neuroendocrinology. Based on his prediction, two research groups led by Roger Guillemin and Andrew Schally contended for the first place to identify hypothalamic hormones including gonadotropin-releasing hormone (GnRH) in 1960s. Their findings of a number of hypothalamic peptides has promoted a better understanding of the endocrine mechanism controlling reproduction. Finally, Guillemin and Schally shared the honor of Novel Prize in 1975 with Yallow who invented the radioimmunoassay. This is a good coincidence to have those 3 scientists shared the honor of the prize and the year was just the beginning of the new mystery for us. What mechanism controls GnRH releae in the brain?

It is well accepted that the surge- and pulse-modes GnRH releases regulate the gonadal activity through controlling gonadotropin release. The surge is generated by a positive feedback action of estrogen and progesterone released from mature follicles. Pulses are fine-tuned by a negative feedback action of ovarian sex steroids. The surge center which is the target of estrogen positive feedback action has been considered to be located in the preoptic area or anterior hypothalamus, because LH surges are induced when estrogen microplants are placed in those brain areas. The localization of pulse-generating mechanism has been much more controversy. Halasz and Pupp first demonstrated that LH pulses are still apparent, after isolating the mediobasal hypothalamus from the rest of the brain with their famous Halasz knife, indicating that a mechanism generating GnRH/LH pulses are located within the mediobasal hypothalamus. Despite a great deal of effort of a number of scientists, brain mechanisms underlying the GnRH surges and pulses and the steroidal feedback action have

been largely unknown for many years. In 2001, a neuropeptide called kisspeptin was found as a ligand for an orphan G-protein-coupled receptor, GPR54 from the human placenta. The discovery of the peptide now provides a clue to neuroendocrinologists to unlock the mystery of the mechanism regulating GnRH release.

A gene encoding kisspeptin had already been found as metastatis-suppressor gene in 1996 and named *Kiss1*. Kisspeptin, therefore, were first considered to be a peptide inhibiting metastatis until GPR54 null mutants were found in two families in Mexico and France by independent two research groups. A lack of GPR54 results in a lack of puberty in both males and females. Administration of kisspeptin shows a profound stimulation of GnRH-dependent gonadotropin release in most mammalian species examined so far. Every initial studies on kisspeptin demonstrated its key role in controlling GnRH release in mammals.

Kisspeptin neurons have two distinct populations in the brain in most mammalian species examined so far. In rodents, one is located in the anterovetral periventricular nucleus (AVPV) or preoptic area (POA), which has been considered to be a center for GnRH surges or target of positive estrogen feedback action. The other kisspeptin population is located in the hypothalamic arcuate nucleous (ARC), which has often been considered to be an essential part of the GnRH pulse generator. Later, the ARC population of kisspeptin neurons became of special interest for the reproductive neuroendocrinologists, because they contain two other peptides, such as neurokinin B (NKB) and dynorphin (Dyn), and therefore referred to as ‘KNDy neuron’. KNDy neurons seem to be equipped with a pulse-generating mechanism, because NKB infusion enhance pulse generation but Dyn inhibits it. Three of theses peptides and their receptors might play key roles in generating GnRH pulses.

We might be close to unveiling the brain mechanism to develop a new concept on the reproduction. The concept would be a key to develop a new approach to control reproduction or treat reproductive disorders. Drugs manipulating the activity of KNDy neurons is now attracting the interest of reproductive scientists or clinicians, who have been looking for a new strategy of the artificial control reproduction.

Early pregnancy recognition - lessons learned from genes expression studies

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Process of maternal recognition of pregnancy (MRP) begins from the appearance of embryo signals and ability of mother to receive those signals leading consequently to maintenance of corpus luteum (CL) and embryo development. According to the old paradigm formation of porcine CL requires only and initial surge of LH and then its further extreme is independent until Day 12 of the estrous cycle, however question whether CL is fully "autonomous" still remains open [1].

In pigs, presence of semen and embryos in the reproductive tract can affect proluteal environment before MRP occurrence. However, after acquirement of luteolytic sensitivity (LS) to $PGF_{2\alpha,}$ CLs regress on Days 15-16 of the estrous cycle. According to previous concepts the MRP in pigs depends mainly on preventing $PGF_{2\alpha}$ access to CL, mediated on the uterine level [2, 3]. However, a potential mechanism by which the conceptuses prevent luteolysis can be based on the change of PGs synthesis in favor of the luteoprotective PGE_2 originating from both conceptuses and endometrium and thus is acknowledged in the pig and other mammalian species an integral part of MRP in the pig and other species. The most important events leading to luteolysis or rescue of CL take place at post- $PGF_{2\alpha}$ and PGE_2 receptor signaling pathways.

During the relatively long absence of luteolytic responsiveness to $PGF_{2\alpha}$ in pig (until Day 12 of the estrous cycle) "luteolytic" $PGF_{2\alpha}$ increases progesterone production in vivo [4] and *in vitro* [5] probably through activation of PKC isoform and then AC/ cAMP/PKA/CREB pathway. After Day 12 of the estrous cycle mediators of luteal regression including TNFα, IFNγ, EDN1 turn the LS "switch" on in the post PTGFR receptor pathway activating IP3/Ca^{++}/RAF/ERK1/2 pathway in the cytoplasm and AP1 in the nucleus as well as PKC in cytoplasm of CL cells. After activation of cFOS and cJUN the functional and structural luteolysis begins.

On the other hand, during the establishment of pregnancy due to action of conceptuses delivered estradiol and PGE_2 the CL "rescue switch" is turned on. PGE_2 through PTGFR2 and PTGER4 receptors may activate PKA leading to the inhibition of main down-stream elements of RAF-ERK1/2 $PGF_{2\alpha}$ signaling pathway (5). Both, PGE_2 receptors where shown to

participate in cAMP production in porcine CL [6]. Moreover, cAMP activate PKA, which affect CREB in nucleus leading to increased luteal steroidogenesis, angiogenesis and survival of CL cells. Additionally, PGE_2 was shown to increase trophoblast adhesion in pigs and other species [7]. These are the main thesis of our "two signal-switch" hypothesis of $PGF_{2\alpha}$ and PGE_2 involvement in regression or rescue of porcine CL. Recently we examined the expression of 70 genes potentially involved in regression or maintenance of porcine CL [7, 9)]. Venn Diagrams revealed that EDN1, CYP19A1, ESR2, JUN and FOS were up regulated on Day 14 of the estrous cycle, whereas among down regulated genes in CL on Day 14 of the estrous cycle in comparison to pregnancy CL KDR, ANGPT2, PTX3, HSD3B1, LDLR, STAR, ESR1, LHCGR, PGR, PGRMC1/2, NR5A1, NFKB1, PTGFS, HPGD were identified.

In silico analysis revealed possible involvement of immune cells in regulation of CL maintenance in the pig and among enhanced processes T cell migration, activation of leukocytes and infiltration of lymphocytes were found. Moreover, increased production of following chemokines, produced by immune components in porcine CL was observed: CXCL10, CXCL9, CCL8 and CXCL2, CCL2, CCL4, CCL5 on Day 12 and 14 of the estrous cycle, respectively [10]. Although the involvement of immune cells in regulation of gene expression and rescue of porcine CL still remains unknown the above presented data suggests potential role of immune system cells in the control of luteal lifespan in the pig.

To characterize transcriptome changes in the porcine endometrium in the course of MPR and initial placentation, several transcriptome analyses using DNA microarray and RNA sequencing (RNA Seq) have been performer [11]. Ostrup et al [12] using the Affymatrix GeneChip Porcine genome Array revealed 263 genes to be differentially expressed (DEG) between the pregnant and non-pregnant sows at day 14 after insemination. In the second study application of Agilent microarrays containing 44 000 oligonucleotide probes, allowed to shows 588 DEGs on Days 15-16 of pregnancy [13]. Whereas Porcine Long oligo microarrays (13 297 probes) revealed only 110 and 139 DEGs on Day 12 and 16 of pregnancy of pregnancy, respectively [14]. All above mentioned studies, among enriched biofunctions and pathways, indicated metabolic process and lipid metabolism, cellular movement, proliferation and cell communication, developmental process, apoptosis, some hormones and growth factors activity were initiated in all three studies as the most altered in pregnant pigs.

While in one study most gene onotology terms significantly enriched at pregnancy had allocated more up-regulated genes then down-regulated in endometrium during early pregnancy [12] the other showed more down-regulated genes compared with the estrous cycle [13]. Interestingly, when RNA-seq method was used, among 35 000 000 sequence reads (per

sample) total 2593 DEGs were determined: with – 1335 genes being expressed at higher and 1258 at lower levels in samples from pregnant gilts compared to non- pregnant controls (15). Using similar method, Zhang et al [16] described a total of 13 612 genes differentially expressed in endometrium between the two breeds (Landrace x Large White and Erhualian) on Day 12 of pregnancy, potentially involved in reproduction and growth.

In the last decade microRNAs (miRNAs) appeared as a new group of regulatory molecules with the potential to degrade mRNA and/or to inhibit protein expression [17]. Recently, miRNAs have emerged as a new players in additional control of embryo development and implantation in mammals via posttranscriptional gene regulation mechanisms. Studying micro RNAome of porcine conceptuses and/or trophoblasts the differential expression of miRNAs on Days 10, 11, 12, 16 and Day 20. Krawczynski et al [18] have found number of the differentially expressed miRNAs between different days. The pregnancy stage-dependent expression profiles of ten genes involved in miRNAs synthesis and transport (DROSHA, DGCR8, XPO5, DICER1, TARBP2, TNRCA, AGO1, AGO2, AGO3 and AGO4) were described in porcine conceptuses and trophoblasts. Additionally, miR-125b was shown to be present in the extracellular vesicle isolated from uterine luminal flushing during pregnancy, suggesting its involvement in diverse biological functions deciding on pregnancy outcome. It seems to be executed through LIF, LIFR and IL-6R action and regulation of endometrial receptivity, immune system, embryo development and implantation, processes and functions identified *in silico*. Therefore it has been concluded that miRNA-125b can be a part of a reciprocal interaction between the conceptus and endometrium.

To characterize complex transcriptomic changes, expression of miRNAs in pregnant and cyclic endometrial collected on Days 12, 16 and 20 were analyzed using Illumina deep sequencing [19]. It was possible to reveal a wide range of miRNAs and isomiRs expressed in the porcine endometrium during the estrous cycle and pregnancy. Worth noting is the fact that isomiRs were shown in work cooperatively with canonical miRNAs to affect gene expression and further biological processes [20]. Bioinformatic analysis suggested a number of miRNA-mediated processes and pathways particularly important for endometrial remodeling and associated with embryo attachment, implantation and placentation. Moreover, in contrast to the estrous cycle several genes involved in miRNA synthesis and transport showed also increased levels in endometrium on Day 16 of pregnancy when compared to Days 12 and 20 (e.g. DROSHA, DICER1), suggesting pregnancy dependent miRNA processing.

Taking together, gene expression studies can give a broad knowledge about the multilevel/multiorgan changes evoked by the signals coming from developing embryo(s).

Using more sophisticated molecular biology techniques it is possible to go deeper into complex process occurring during periods critical for pregnancy outcomes in many mammalian species. Although identified genes may have conserved roles during the establishment of pregnancy in mammals and reflect basic principles of mammalian reproduction we need to bear in mind that several posttranscriptional mechanisms, involving e.g. miRNAs, can affect execution of final functions in the cell. Thus, caution conclusions need to be drawn from transcriptomic data. However, in some cases gene expression studies can be excellent support or weakness of previously developed/published theories and mechanisms.

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Molecular mechanisms involved in segregation of inner cell mass and trophectoderm lineages in bovine and porcine embryos

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Successful offspring production from *in vitro* produced embryos, such as *in vitro* fertilization (IVF) or somatic cell nuclear transfer, has accelerated progress in these areas. However, *in vitro* production (IVP) of bovine or porcine embryos is still inefficient compared with that of other mammals, such as mice. One of the reasons for the decreased development of the bovine or porcine IVP system is limited knowledge concerning the molecular mechanisms involved in early embryonic development. Therefore, to improve the IVP system for bovine and porcine embryos, it is important to focus on the molecular mechanisms underlying the regulation of early embryonic development.

In mouse embryos, differentiation of inner cell mass (ICM) and trophectoderm (TE) is regulated by various transcription factors, such as Oct-4 and Cdx2, but molecular mechanisms that regulate differentiation in bovine and porcine embryos remain unknown. To evaluate gene transcripts involved in segregation of ICM and TE lineages in bovine and porcine embryos, we examined the relative abundances of *OCT-4* and *CDX2* transcripts in these embryos. In bovine and porcine embryos, *OCT-4* transcript levels in ICM lineages were significantly higher than that in TE lineages. In contrast, the *CDX2* levels were lower in ICM lineages than that in TE lineages. These findings suggested that OCT-4 and CDX2 are involved in differentiation of ICM/TE linages in bovine and porcine embryos.

To clarify the necessity of OCT-4 for functional characterization in bovine and porcine embryos, we attempted OCT-4 downregulation of early embryos by RNA interference. My collaborator, Nobuyuki Sakurai will present results of OCT-4 downregulation in bovine embryos in the same session in this meeting. My presentation in the session focus on the role of OCT-4 for the segregation of ICM and TE of porcine preimplantation embryos. The relative abundance of *Oct-4* in embryos injected with OCT-4 siRNA was significantly ($P < 0.05$) lower than that in uninjected and control siRNA-injected embryos. Expression of the Oct-4 protein was evaluated by immunofluorescent staining. Although the Oct-4 signal was also observed in nuclei of OCT-4 siRNA-injected embryos, the signal intensity was weaker

than that observed in uninjected and control siRNA injected embryos. Thus, knockdown of Oct-4 by siRNA-2 was effective until at least the morula stage. *In vitro* developmental competence of OCT-4 siRNA-injected embryos was evaluated (Table 1).

Table 1. Effect of Oct-4 siRNA injection on *in vitro* development of porcine embryos.

Treatment	Number of embryos cultured	No. (%)[†] of embryos developed to			
		Day 2	Day 4	Day 5	Day 6
		2-cell ≤	Morula	Blastocyst	Blastocyst
Uninjected	244	130 (53.3)	47 (19.3)	60 (24.6)[a]	57 (23.4)[a]
Control siRNA	237	130 (54.9)	44 (18.6)	45 (19.0)[a]	42 (17.7)[a]
OCT-4 siRNA	237	120 (56.0)	44 (18.6)	11 (4.6)[b]	17 (7.2)[b]

* Experiments were replicated five times; [†] Percentages of the number of embryos cultured.[a, b] Values with different superscripts within each column differ significantly ($P < 0.05$).

The OCT-4 siRNA-injected embryos developed to the morula stage on day 4 (day 0 = IVF), and there was no significant difference between the experimental groups (18.6 – 19.3%). However, on both day 5 and day 6, the blastocyst developmental rates of OCT-4 siRNA-injected embryos (4.6 and 7.2%, respectively) were significantly ($P < 0.05$) lower than those of the uninjected (24.6 and 23.4%, respectively) and control siRNA-injected embryos (19.0 and 17.7%, respectively). Representative photographs of embryos morphology are shown in Fig. 1. In the uninjected and control siRNA groups, the morula embryos developed to the blastocyst stage on day 5, and these embryos were expanded on day 6. However, the OCT-4 siRNA-injected embryos showed developmental arrest at the morula stage, with only a few embryos reaching the blastocyst stage. Moreover, almost all embryos injected with OCT-4 siRNA were collapsed structures at day 6. Namely, porcine embryos with downregulated Oct-4 expression failed to segregate both ICM and TE lineage from the morula stage.

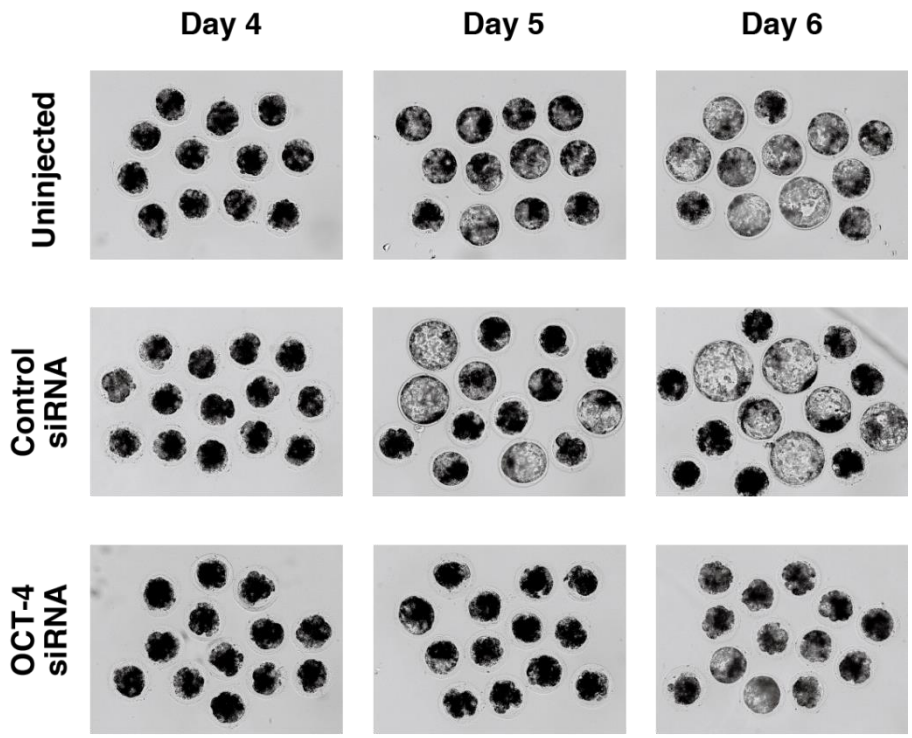

Fig.1. Representative photographs showing the dvelopmental morphology of porcine embryos obtained with or without siRNA injection.

To clarify the necessity of OCT-4 for segregation of TE lineage in porcine embryos, we investigated the developmental morphology of porcine embryos constructed with OCT-4 siRNA-injected and uninjected blastomeres. OCT-4 siRNA and TRITC-dextran (fluorescent dye) conjugate was injected to one blastomere of 2-cell stage porcine embryos obtained from parthenogenetic treatment. Interestingly, some blastomeres derived from OCT-4 siRNA injection contributed to the TE linage. However, almost blastomeres derived from OCT-4 siRNA injected degenerated. This result suggested that OCT-4 directory expression in the blastomere is essential for TE formation in porcine embryos.

In conclusion, our results indicated that OCT-4 is a key factor for differentiation of both ICM and TE linages in porcine embryos. The present study is the first to demonstrate the critical importance of Oct-4 for early development of porcine embryos and may also provide the basis to understand the mechanism of early lineage segregation in porcine embryos.

The effect of Wnt/β-catenin signalling on bovine preimplantation development – prospects for bovine ESC derivation

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Despite the advances in the stem cell research and almost 20 years after the establishment of the first bovine embryonic stem cell lines (bESC), stable true ESC from farm animals still remain elusive. Bovine embryos present a very promising model, not only for animal biotechnology and medicine, but also for investigating the fundamental mechanisms of early human development. The similarities include the timing of epigenetic reprogramming, the stage of the embryonic genome activation and the timing of development [1]. However, the classic mouse and human ESC derivation protocols have proven unsuccessful for the majority of mammals. The existing ungulate ESCs exhibit several deficiencies such: as short live in culture, inability to contribute to the chimeras, failure to achieve the germline transmission. This may arise from the genetic background of the animal, differences in embryogenesis, gene expression patterns and embryonic lineage specification. Thus, in order to understand the underlying mechanisms of pluripotency, our research concentrates on unravelling the basic processes governing embryonic cell fate decisions.

The results of our work indicate the existence of lineage specific differences in the expression pattern of key pluripotency markers in bovine inner cell mass (ICM) and trophectoderm (TE), both at the mRNA and protein level [2]. The specific lineage segregation is visible as OCT4 and NANOG becomes ICM specific and CDX2 locates in the TE. These early cell fate decisions are governed by the interactions of numerous signalling pathways and the downstream action of the transcription factors.

The studies of species such as mouse, rat and human indicate the WNT pathway as one of the key pluripotency regulators in mammals. Therefore, we aimed to investigate the effect of WNT signalling (by GSK3 inhibition) during the preimplantation period of bovine development, and to verify the subsequent potential of primary ICM outgrowths to support bESC maintenance in culture. The precise effect of the WNT activity during the development of cow embryo has not been studied in detail. The overall conclusion is that the WNT pathway is present and active in bovine embryos, and that it influences development, but the

actual correlation between the WNT activity and pluripotency potential of the ICM and bESC has not been looked at. This is a novelty arising from our work. We believe, that before testing the ESC culture conditions that have proven successful for the model species and switching to the combination of the inhibitors (such as the 2i/3i systems [3]) it is crucial to understand the roles of the signalling pathways independently of one another. It is especially important, since depending on the species, the GSK3i action may be enhanced by different factors (LIF for mouse and rat and FGF for human).

Thus, we have investigated the effect of WNT activation on pluripotency marker gene expression in the ICM and the TE and studied the derivation potential of primary bESC lines from blastocysts obtained in the presence of the GSK3i. WNT activity exerted a positive effect on pluripotency gene expression in developing bovine embryos, manifested by up-regulation of OCT4, NANOG, REX1, SOX2, c-MYC and KLF4 in the ICM and down-regulation of CDX2 in the TE (Fig. 1).

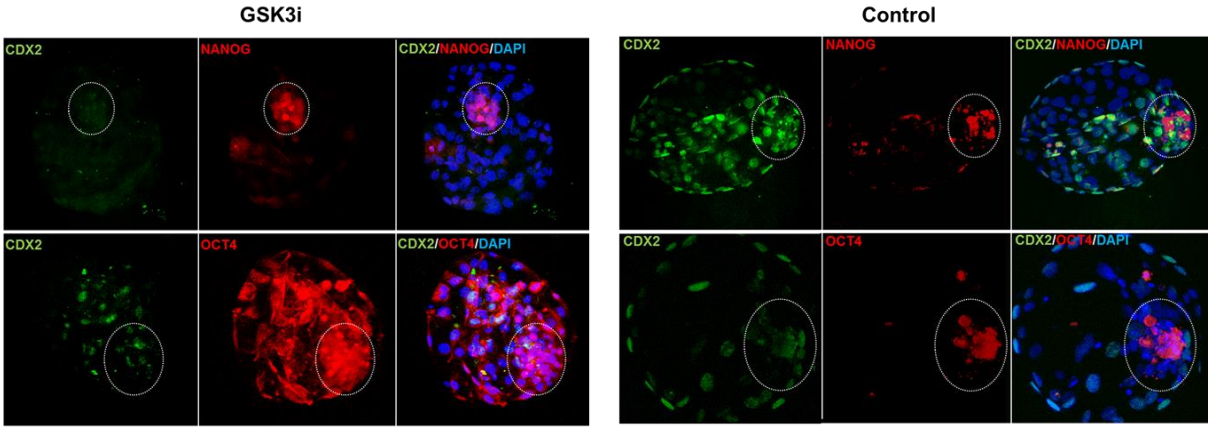

Figure 1. Pluripotency marker distribution in bovine blastocysts after GSK3i supplementation of in vitro embryo culture system. Left panel: CDX2 distribution changes (loss of TE specific expression pattern), OCT4 up-regulation both in the ICM and the TE, NANOG remains ICM specific. Right panel - the controls show the lineage specific expression pattern of OCT4 and NANOG in the ICM and CDX2 in the TE of blastocysts obtained w/o the inhibitor. The encircled area indicates the ICM. DAPI marks the chromatin. Images from Madeja et. al., 2015 (ahead of print [4]: online at www.libertpub.com/scd).

The results of bESC derivation experiments allowed us to speculate that the derived cell lines may share features of both naïve and primed ESCs. Similarly to mouse epiblast stem cells and human ESC the derived lines grew as flat, mono-layer colonies intolerant to passaging as single cells (Fig. 2). JAK/STAT signalling was indispensable for proper colony formation and proliferation, yet LIF alone was inefficient to support self-renewal. Concomitant with the naïve state of mouse ESC, WNT activity supported by LIF had beneficial effects on cell

culture propagation, survival after passage, morphology and pluripotency related marker gene expression [4].

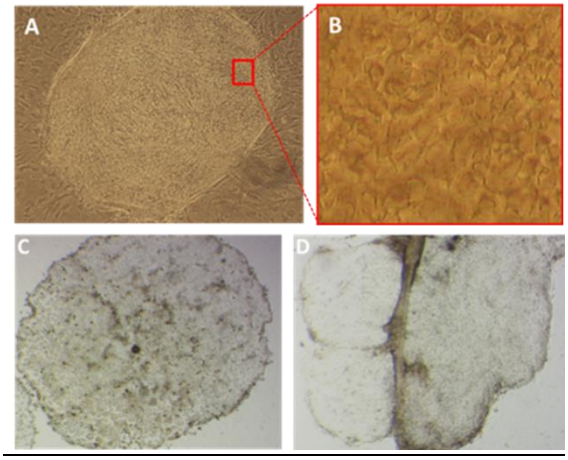

Figure 2 Example images of bESC-like colonies. **(A)** depicts +LIF + GSK3i derived colony, **(B)** is a magnification of the boxed area. The uniform, single layer of cells without signs of differentiation is visible. **(C)** and **(D)** indicates differentiated colonies. **(C)** - colonies derived w/o the GSK3i; **(D)** - differentiated colony, a result of culture condition change after passage (P) =3. The colonies were initially cultured in +LIF+ GSK3i, after P=4 LIF and GSK3i were withdrawn.

Founding: NCN-4429/B/P01/2010/39 and FNP- HOM/2009/B

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How is oviductal motility controlled?: Production mechanisms of local factors which regulate smooth muscle contraction and relaxation in cattle

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Oviductal motility is required for transport of oocyte and embryo, resulting in successful fertilization and implantation in mammals. An ovulated oocyte is transported to the ampulla, followed by fertilization within a day after ovulation. Then, the fertilized egg passes through the isthmus and reaches the uterus at 3-4 days after ovulation. The oviduct consists of epithelial, stromal and smooth muscle layers. Oviductal epithelial and stromal cells produce various factors that contribute to regulation of oviductal functions. The smooth muscle layer contains longitudinal and circular muscles, which work in a coordinated manner to transport the oocyte and the embryo. Oviductal smooth muscle motility is systemically and locally regulated by various factors including prostaglandin F2 alpha (PGF) and endothelins (EDNs) as contractile factors, and prostaglandin E2 (PGE) and nitric oxide (NO) as relaxant factors [1-5]. The contents of PGs in the bovine oviductal tissues are highest at peri-ovulation period [5]. In addition, we have recently found the mRNA expressions of inducible NO synthase (iNOS), *EDNs* and *EDN converting enzymes (ECEs)* are highest at day of ovulation in the ampullary tissue of bovine oviduct. The objective of our research is to clarify the regulatory system of oviductal motility including the production mechanisms of these factors in cattle.

First, we developed the isolation and culture systems of bovine oviduct epithelial and stromal cells to examine their functions. The homogeneities of stromal and epithelial cells, based on immunofluorescent staining, were higher than 99%. Subsequently, the factors which influence on the expressions of PGs, EDNs, ECEs and iNOS were investigated using these new established methods.

Prostaglandin production

Both epithelial and stromal cells of the bovine oviduct produced PGs. Tumor necrosis factor alpha (TNF), a cytokine produced by the bovine oviduct, stimulated PGF production by cultured epithelial and stromal cells. Lysophosphatidic acid (LPA), one kind of phospholipids, stimulated cyclooxygenase (COX)-2 expression and PG production by only isthmic stromal cells. This region-specific effect of LPA may relate to difference of LPA receptor (LPAR) expression, since mRNA expressions *LPAR4-6* were significantly higher and *LPAR1-3* were significantly lower in the isthmus compared to the ampulla.

Nitric oxide synthase expression

Regulatory factors of *iNOS* expression were investigated using the cultured epithelial cells. Estradiol-17β (E2:0.1, 1, 10 nM) suppressed *iNOS* expression in isthmic epithelial cells after 24 h incubation. The inhibitory effect of high dose E2 (1000 nM) on *iNOS* expression was also observed after short-term incubation (1 h). The *iNOS* expression in ampullary epithelial cells was also regulated by the same concentration of PGs with follicular fluid. Both PGF and PGE stimulated *iNOS* after 1 h incubation, while PGs suppressed *iNOS* expression after 24 h incubation.

Endothelin expressions

EDN and *ECE* expressions were regulated by ovarian steroids in cultured epithelial cells. Estradiol-17β (1, 10 nM) stimulated *EDN1, EDN2* and *ECE1* expressions, and progesterone (10, 100 nM) stimulated ECEs expression in only ampullary cells after 4 h incubation. On the other hand, high E2 (100 nM) stimulated *EDN2* expression within a short time (0.5 and 1 h) in isthmic cells. Since there are several kinds of estrogen receptors, we hypothesize that the region-specific effects of E2 are due to different estrogen receptors.

In summary, the productions of regulators for smooth muscle motility seem to be controlled by several factors originated from circulation, follicular fluid which enters oviduct with an oocyte at ovulation, and oviductal secretion in the bovine oviduct (Figure 1). Furthermore, our results suggest that the effects of each local factor differ between the ampulla and isthmus in the same types of cultured cells. It suggests that regulators of smooth muscle contraction and relaxation are produced during the optimal period and at proper location to transport the oocyte and early embryo in the bovine oviduct. Although the precise control of oviductal motility is essential for successful pregnancy, methods for diagnosing and treating of its functional abnormality have not been established yet not only in cows but also in other animals. Our studies should contribute to improving the fertility rates in mammals.

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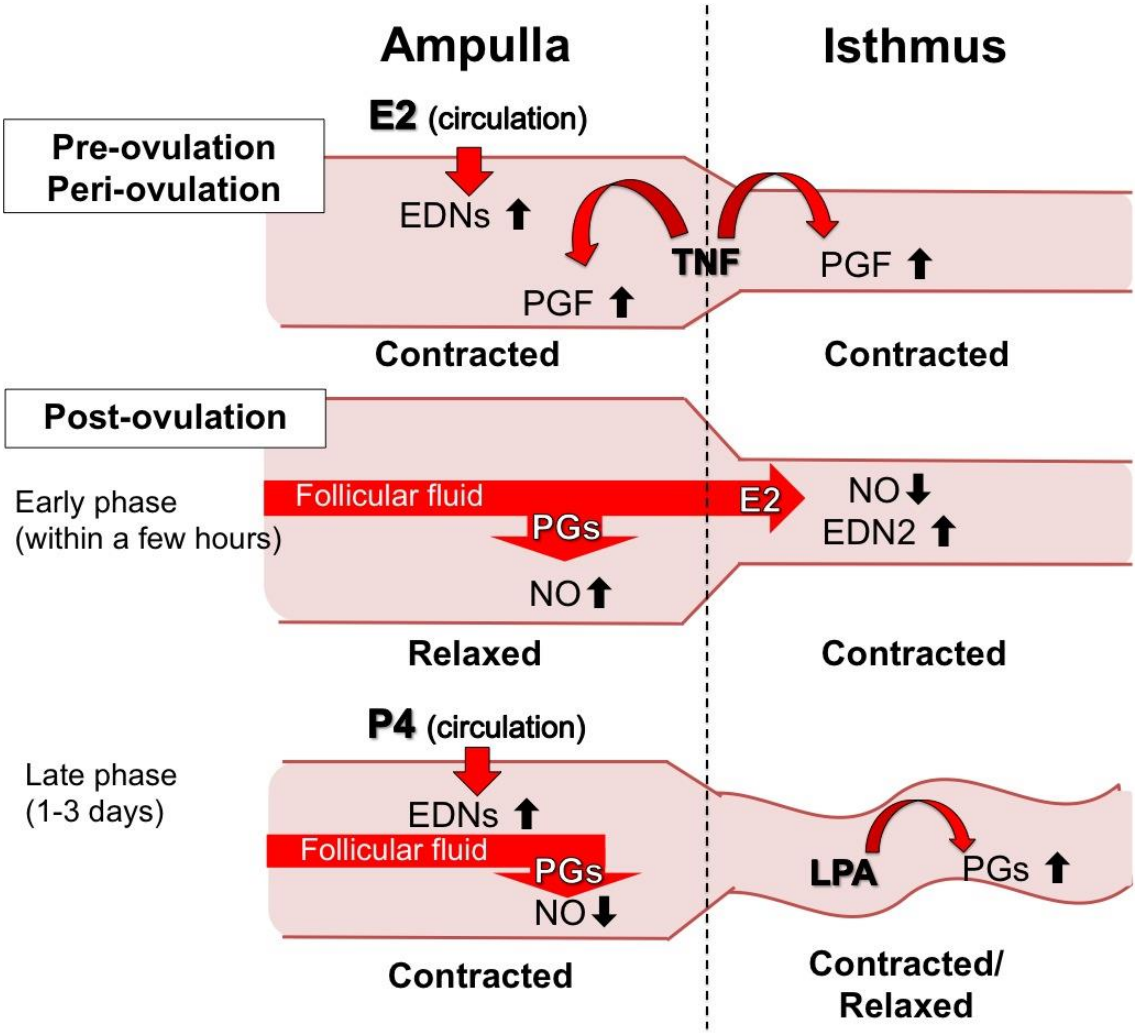

Figure 1. Hypothesis derived from our results on production mechanisms of local regulators for smooth muscle contraction and relaxation in the bovine oviduct. Pre-ovulation period: Estradiol-17β from circulation increase EDN production in the ampulla. Peri-ovulation period: Tumor necrosis factor alpha from oviductal secretion increases PGF production in the ampulla and isthmus. Post-ovulation period: Prostaglandins from follicular fluid increase NO production in the ampulla, and high E2 from follicular fluid decreases NO and increases EDN2 production within a few hours after ovulation. One-three days after ovulation, progesterone from circulation facilitates EDN production and PGs from follicular fluid decrease NO production in the ampulla. In addition, LPA from oviductal secretion increases both PGF and PGE production in the isthmus.

Functional morphology of cattle oviduct: comparison of morphology of bovine epithelial cells (BOECs) at an elevated temperature

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The oviduct is responsible for many functions such as: catching oocytes; migration of oocytes, sperm and embryos; fertilization and early embryo development [1]. The system of *in vitro* culture of bovine oviduct epithelial cell (BOEC) aggregates with embryos can be used to determine the mother-embryo interaction in many aspects [2]. One of the interesting issues is the influence of elevated temperatures on the oviduct [3], especially on bovine oviduct epithelial cells (BOECs), which are formed by ciliated and secretory cells [4].

The objective of this study was to evaluate the ultrastructure of BOECs cocultured with cattle embryos at the elevated temperature of 41°C.

Bovine oviducts and ovaries were collected *post mortem* from slaughtered cattle. Oviducts were obtained from cattle in phase I of the ovarian cycle (between 0 and 4^{th} day, where day 0 means ovulation) based on the ovarian morphology. Bovine oviduct epithelial cells were mechanically isolated from the oviducts and cultured for 48 hours at control temperature (38.5°C) for creation BOEC aggregates (Rottmayer et al. 2006). These BOECs were cocultured with cattle embryos obtained *in vitro* at the control (38.5°C) and elevated (41°C) temperatures for 168 hours. After coculture, samples of BOECs from both groups were taken for analysis: 1) viability (Trypan blue test), 2) cilia movement (subjective evaluation) and 3) ultrastructure (SEM, TEM methods) of: a) secretory cells (length of microvilli, number of granules) and b) ciliated cells (length of cilia, number of basal bodies). Additionally, the embryo development was analysed. Statistical analysis was performed by Statgraphics 5.0 Centurion using T-test for calculation of the significant differences between the temperatures. Difference at $P<0.001$ was considered significant.

At the control temperature (38.5°C) the embryos cocultured with BOECs developed better than at elevated temperature (41°C), which was demonstrated by a significantly higher percentage of cleaved embryos (84.30±2.76 vs 62.25±1.73) after 48 hours and blastocysts (30.03±2.07 vs 0) after 168 hours ($P<0.001$). After 168 hours, percentage of viable cells was similar in BOECs cultured with embryos at control temperature and elevated temperature.

Analysis of the cilia movement showed vigorously beating cilia on BOECs aggregate surfaces cultured both at control and elevated temperatures. The SEM micrographs' analysis of the BOEC aggregates indicated that the ultrastructure of cells surface in BOEC aggregates cultured at elevated temperature was similar to BOECs cultured at control temperature. The length of cilia on BOECs surface at control temperature (5.5 μm±0.3) was similar to elevated temperature (5.3μm±0.2). The number of microvilli on secretory cells was also similar either at control (44.5±1.3) or elevated (43.7±1.2) temperatures. The TEM micrographs' analysis of secretory granules indicates their location in apical part of secretory cells. The number of secretory cells of BOECs cultured at control temperature (12±3.6) was similar to elevated temperature (11.9±4.1) and also their ultrastructure was similar. The ultrastructure analysis of basal bodies in ciliated cells also indicates no differences between control and elevated temperatures.

These results indicate that elevated temperature of 41°C inhibits the cattle embryos development, which is corresponded with previous studies [5].

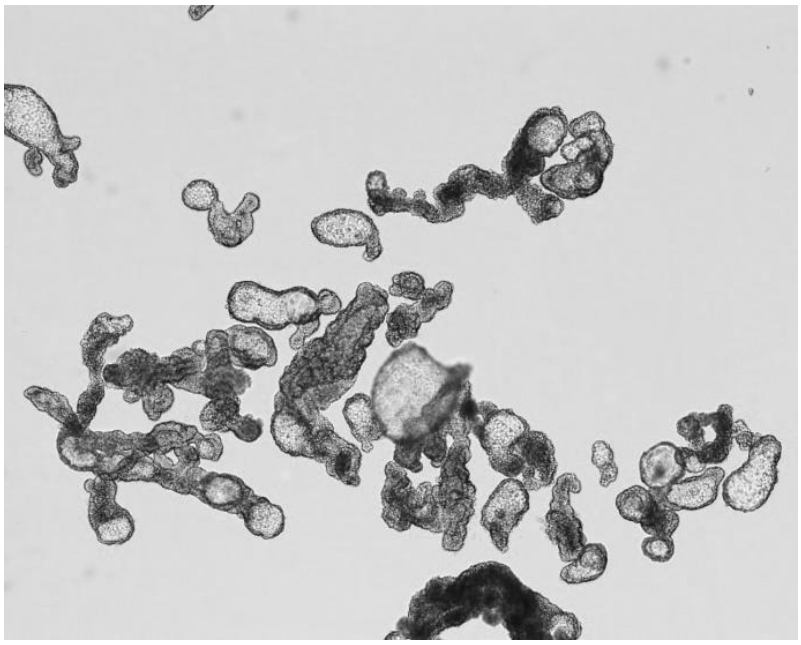

Figure 1. Light microscopic image of BOECs after 168 hours coculture with cattle embryos at elevated temperature of 41°C (63x).

Interestingly, that elevated temperature has no effects on the viability, cilia movement and morphology of BOECs. The maintenance of the correct morphology (Fig.1, Fig.2), especially granules in secretory cells and cilia with their basal bodies, testifies to the high resistance of these cells to heat shock. In conclusion, the elevated temperature has no effect on the morphology of BOECs but negatively influences cattle embryo development.

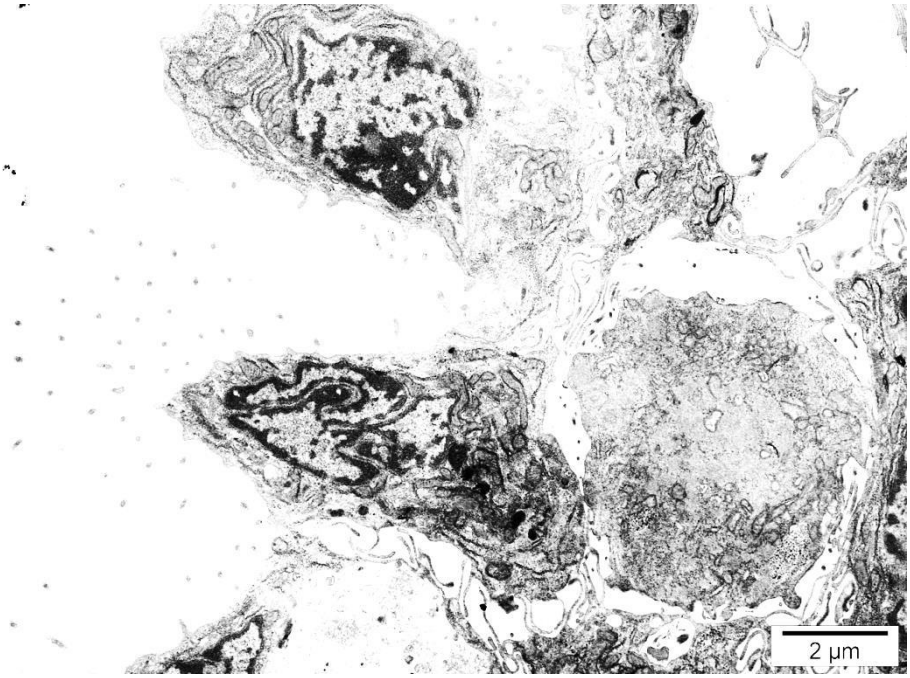

Figure 2. TEM image of ciliated and secretory cells after 168 hours coculture with cattle embryos at elevated temperature of 41°C. Bar = 2µm.

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Changes in the gene expression profiles of bovine corpus luteum during early pregnancy

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Although regulation of corpus luteum (CL) function throughout the estrous cycle has been intensively studied, studies of CL function during the entire gestation period are limited. Understanding the role of CL function during pregnancy might help to determine a way to improve reproductive efficiencies and reduce the number of defective fetuses. Therefore, in the present study, to evaluate functional changes of the CL during early pregnancy in cows, global gene expression profiles of the CL at the time of maternal recognition were investigated.

Bovine ovaries containing CLs were obtained from the Japanese Black cows on days 15 and 18 after artificial insemination, and a presence or absence of conceptus was checked macroscopically to determine whether the cows are pregnant or not. The day of artificial insemination was designated as day 1. The CL tissues were immediately separated from the ovaries and then cut into small pieces. These CL pieces were submerged in RNAlater or 10% neutral formalin, and stored until use for gene expression analysis and histological analysis, respectively. All procedures for animal experiments were carried out in accordance with guidelines approved by the Animal Ethics Committee of the National Institute of Agrobiological Sciences for the use of animals.

Microarray analysis, using a custom-made 15 K bovine oligo DNA microarray (platform GPL9284)[1], demonstrated 30 and 266 differentially expressed genes in the CL on days 15 (P15) and 18 (P18) of pregnancy compared with the CL on day 15 (NP15) of non-pregnancy (Table 1, n=4 for each group, >2-fold change relative to NP15; $P<0.05$). The gene expressions of *peroxisome proliferator-activated receptor delta (PPARD)* were the highest and *cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2)* were the lowest in both the pregnant CL (P15, P18) compared with the non-pregnant CL (NP15), and these microarray results were validated by quantitative real-time PCR analysis (Fig. 1, $P<0.05$). Immunohistochemical staining showed that PPARD (1:50, ARP38765_T100, Aviva Systems Biology) was clearly expressed on the nuclei in both the luteal and endothelial cells and that

CYP21A2 (1:25, ARP60144_P050, Aviva Systems Biology) was expressed on the cytoplasm of luteal cells. In addition, transcripts of interferon-induced genes (*ISG15, MX1 and OAS1)* were more abundant in the pregnant CL (P18) than non-pregnant CL (Fig. 2, $P<0.05$), and both the mRNAs and proteins for type I interferon receptors (IFNαR1 and IFNαR2) were expressed in bovine CL cells of the estrous cycle and pregnancy.

Table 1. Top 10 genes of increased or decreased in the CL of pregnancy (P15 and P18) compared with the CL of non-pregnancy (NP15)

Day 15 of pregnancy (30 genes)			**Day 18 of pregnancy (266 genes)**		
Gene	**Accession No.**	**Fold**	**Gene**	**Accession No.**	**Fold**
(Increased)			(Increased)		
PPARD	*NM_00108363*	6.61	*PPARD*	*NM_001083636*	15.9
MSX2	*NM_00107961*	5.84	*CD5L*	*NM_001102119*	11.9
PFN3	*NM_00107794*	5.31	*ERCC2*	*NM_001103317*	11.8
MRPS28	*NM_00104640*	4.63	*ISG15*	*NM_174366*	8.32
NUCB1	*NM_00107519*	3.66	*ZNRD1*	*NM_001046400*	7.50
SSPO	*NM_174706*	3.17	*MSX2*	*NM_001079614*	5.08
TERF2IP	*NM_00107541*	3.13	*P2RY4*	*AY540307*	4.25
LAS1L	*NM_00107681*	3.11	*NOP10*	*NM_001114521*	4.04
ITGB5	*NM_174679*	3.09	*LMO3*	*NM_001046337*	3.72
PPP2R4	*NM_00104617*	2.96	*GJB1*	*NM_174069*	3.57
(Decreased)			(Decreased)		
CYP21A2	*NM_174639*	5.10	*CYP21A2*	*NM_174639*	6.16
SCG2	*NM_174176*	3.30	*CSNK1D*	*XM_002687933*	4.87
EDEM3	*NM_00120585*	2.68	*PLCG1*	*NM_174425*	4.55
PRF1	*NM_00114373*	2.61	*FN1*	*NM_001163778*	4.30
HSD3B1	*NM_174343*	2.60	*ANKRD50*	*NM_001205949*	4.17
UTS2R	*NM_00104048*	2.52	*HSPA1A*	*NM_174550*	3.87
TPD52L1	*NM_00107603*	2.36	*WRNIP1*	*NM_001103106*	3.85
POLR1B	*NM_00110029*	2.24	*EIF4B*	*NM_001035028*	3.45
LRSAM1	*NM_00107529*	2.17	*PPP4C*	*NM_001099108*	3.41
SF3A2	*NM_00109921*	2.10	*RPA1*	*NM_001075176*	3.34

Collectively, the preceding results demonstrate that the number of genes whose expressions changed in the CL during the transition from the estrous cycle to pregnancy gradually increased with the stage of gestation. This suggests that the different gene expression profiles may contribute to functional changes of the bovine CL during early pregnancy, and that embryonic signals, *e.g.*, interferon-τ may act on the CL by a counter-

current mechanism. The substances that exhibit changes in their expression levels in the pregnant CL may play a role(s) in regulating bovine CL function at the time of maternal recognition. Since PPARD induces progesterone (P4) production by stimulating cholesterol uptake in steroidogenic cells [2] whereas CYP21A2 enzymatically catabolizes P4 to deoxycorticosterone [3], high levels of PPARD and low levels of CYP21A2 expressions in the early pregnant CL may contribute to support P4 production by bovine luteal cells.

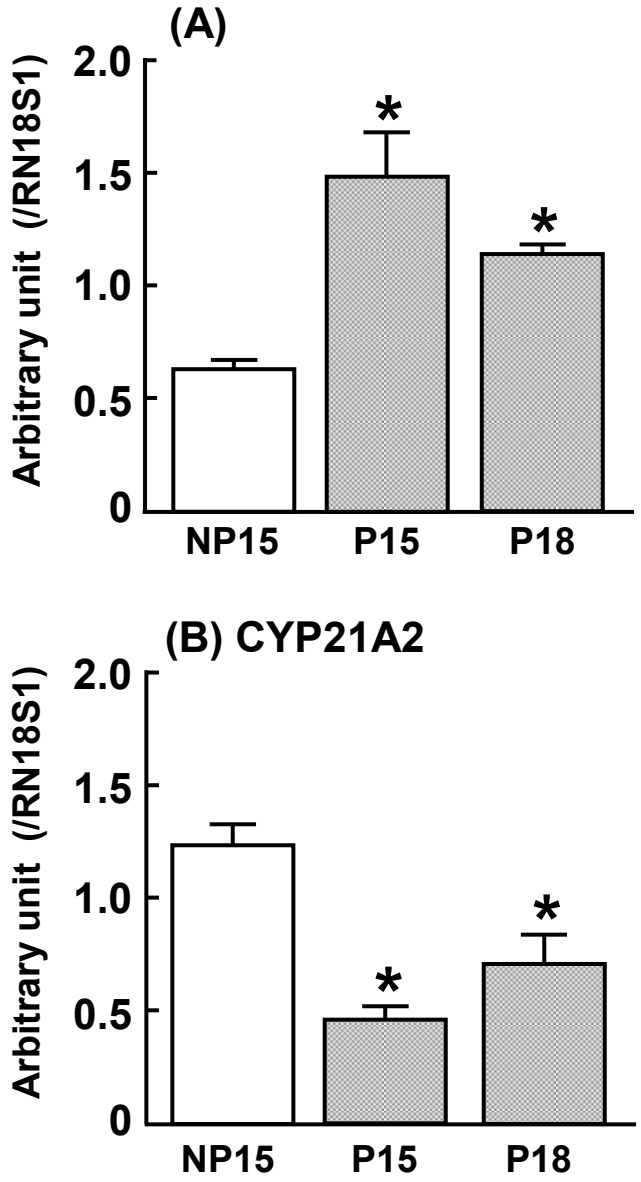

Fig. 1. Changes in relative amounts of mRNAs for (A) *PPARD* and (B) *CYP21A2* in the bovine CL of Day 15 (P15) and Day 18 (P18) of pregnancy compared with the CL of non-pregnancy (NP15). Data are means ± SEM for four cows per stage and are expressed as relative ratios of the mRNAs to *18S ribosomal RNA* (*RN18S1*). Asterisks show significant differences between non-pregnant and pregnant CL (*$P < 0.05$ or lower).

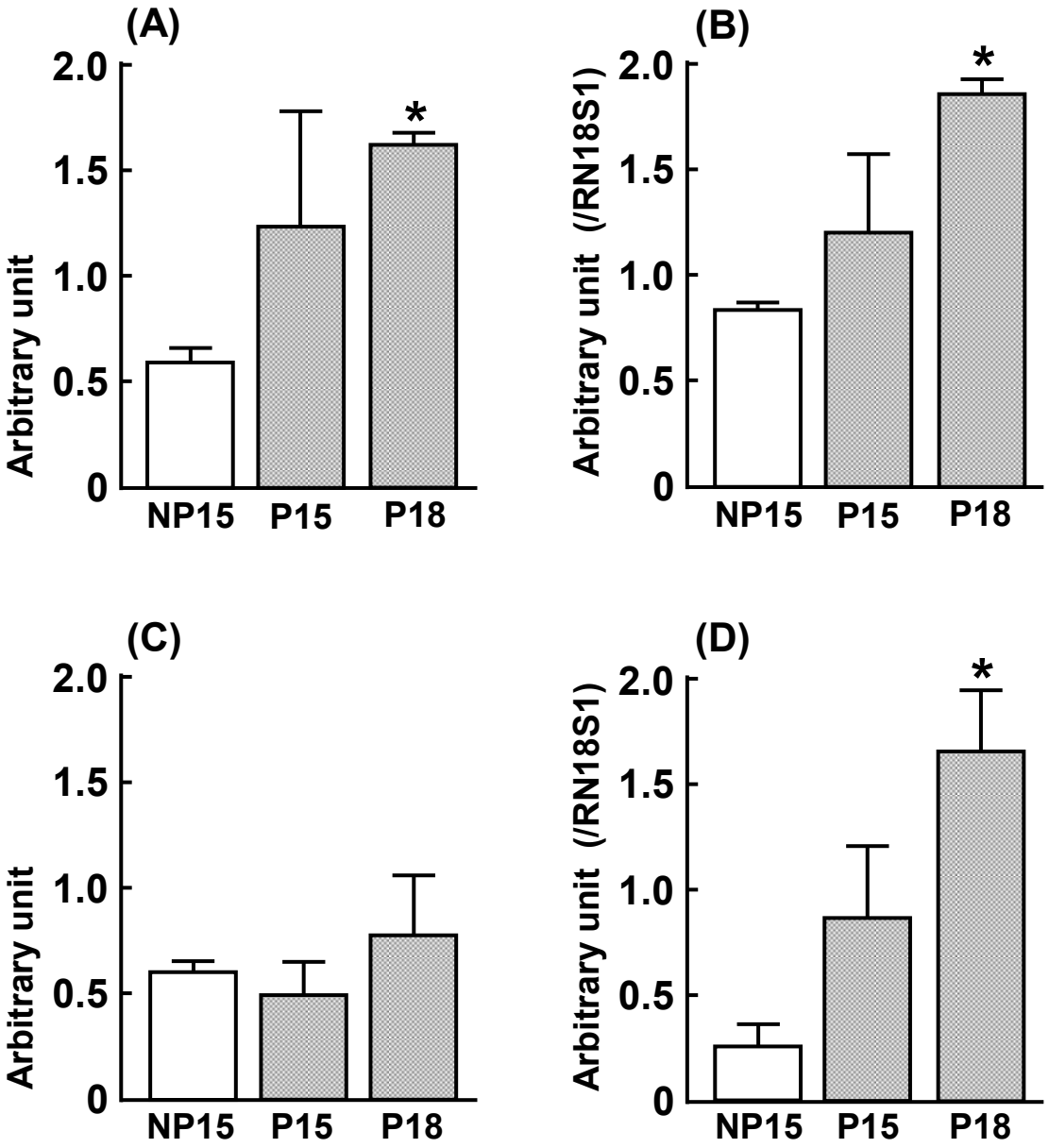

Fig. 2. Changes in relative amounts of mRNAs for (A) *ISG15*, (B) *MX1*, (C) *MX2* and (D) *OAS1* in the bovine CL of Day 15 (P15) and Day 18 (P18) of pregnancy compared with the CL of non-pregnancy (NP15). Data are means±SEM for four cows per stage and are expressed as relative ratios of the mRNAs to *18S ribosomal RNA* (*RN18S1*). Asterisks show significant differences between non-pregnant and pregnant CL (*$P < 0.05$ or lower).

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Evaluation of an alternative embryo transfer strategy to mitigate early embryonic loss and differential gene expression in endometria of fertility and sub-fertile cattle

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Over the last few decades reproductive research has focused on reversing the declining pregnancy rates that negatively affect calf and milk production, due to the significant economic losses for livestock producers. Consequently, numerous studies have sought to improve reproductive performance however few, if any, have identified truly effective solutions. Here this study aimed to determine the critical period of embryo loss after embryo transfer (ET) and investigate whether implementation of an alternative embryo transfer strategy could mitigate early embryonic loss. Moreover, it examined whether endometrial gene expression differs with respect to fertility status in the early pregnancy period.

In the first study, pregnancy loss was monitored following ET on day 7 post-estrus in Japanese Black cows (n=466). In total 52% (n=242) of the recipient cows failed to establish a pregnancy, with 61% (n=148) of these cows returning to estrus within a normal cycle length (< 25 days). In a second study, the timing and ability to detect early pregnancy loss was investigated by the measurement of *interferon stimulated gene* (*ISG*) 15 expression levels in peripheral blood mononuclear cells (PBMCs) of non-pregnant and pregnant cows. Previous studies have identified interferon-tau (IFNτ) secretion by the conceptus effuses into the circulation and increases *ISGs* expression in PBMCs [1]. From the cohort of cows subjected to a day 7 ET in the first study, PBMCs were collected on days 16, 18 and 21 post-estrus from pregnant cows (n=16), cows that failed to establish a pregnancy and returned to estrus < 25 days (n=17) or ≥25 days (n=11), as well as cows in which no ET had been performed (cyclic cows, n=15). RNA was extracted from all samples, cDNA synthesized and the expression of *ISG15* analyzed by quantitative PCR. On day 18 post-estrus, *ISG15* expression was similar between cows that returned to estrus <25 days and cyclic cows. Compared with both these groups, *ISG15* expression was significantly ($P<0.05$) higher in pregnant cows. The expression level of *ISG*15 in cows returned to estrus ≥25 days showed the intermediate value between pregnant and cyclic cows or cows that returned to estrus <25 days. These results suggest that the embryos of the cows that returned to estrus <25 days (the vast majority) fail to secrete

sufficient IFNτ in the period of maternal recognition of pregnancy to alter *ISG15* expression in PBMCs. Thus, these embryos likely die quickly after ET on day 7.

To test this hypothesis a third study investigated the transfer of an older day 14 conceptus could bypass the critical period for embryo survival resulting in an improved pregnancy rate. A non-surgical conceptus transfer (CT) method was developed, in which day 14 conceptuses were recovered by non-surgical flushing of uteri of superovulated cows [2]. The presence of visible embryo disc was confirmed and an individual conceptus was non-surgically transferred to the uterine horn ipsilateral to the CL of a day 14 synchronized recipient known to be either sub-fertile (defined by a failure to establish a pregnancy following more than three standard ETs; n=42) or fertile (had previously established a pregnancy following less than three standard ETs; n=33). Ultrasonography on day 80 confirmed CT pregnancy rates were 2.4% in sub-fertile and 54.5% in fertile cows. These values were comparable to standard ET (subf-fertile: 5.6%, fertile: 47.4%). This failed to support the hypothesis that transfer of an older embryo, in order to surpass the critical period for embryo survival seen in ET on day 7, could improve pregnancy rates.

In a fourth study, differences in endometrial function were investigated between fertile and sub-fertile cows. Intercaruncular endometria were biopsied from the ipsilateral uterine horn of fertile (n=6) and sub-fertile (n=5) multiparous cows on day 7 post-estrus. RNA was extracted from samples and gene expressions were determined by microarray (Agilent, 43k probes). Microarray data analysis was performed by GeneSpring. The results showed that more genes were induced expression in sub-fertile cows compared with fertile cows ($P<0.05$, fold change$\geq$2). According to hierarchical cluster analysis, all cows are separated into two groups. One group was composed of only fertile and the other was sub-fertile cows. Taken together, these data support that even early in the post-estrus period the endometrial gene expression differs substantially between fertile and sub-fertile cows.

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The effect of lysophosphatidic acid (LPA) on the embryo-maternal cross-talk in cows

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Lysophosphatidic acid (LPA) is a simple phospholipid with a vast variety of physiological and pathological actions on many cell types. In mammals, LPA exerts its action via several high affinity G-protein-coupled receptors (LPARs). So far many studies have been conducted on the influence of LPA on the regulation of reproductive processes in various animal species including cow. We found that LPA stimulated the insemination rate in cattle as well as progesterone (P4) and luteotropic prostaglandin (PG)E_2 secretion during estrous cycle and early pregnancy *in vivo* and *in vitro*. The presence of LPA as well as enzymes responsible for LPA synthesis and specific LPARs in the bovine endometrium, CL, follicle, oocyte and embryo was detected, indicating that these structures of the bovine reproductive tract are the sites of LPA synthesis and targets for its action during the estrous cycle and early pregnancy. However, during the estrous cycle and early pregnancy the most important place for LPA synthesis seems to be endometrium while the ovary and the embryo the most important targets for LPA action. We documented that LPA exerted auto/paracrine actions in the bovine reproductive tract. In the bovine CL, the luteotropic action of LPA resulted from its effect on the augmentation of P4 synthesis via the stimulation of 3βHSD expression. Moreover, we found LPA-dependent stimulation of IFNτ action on 2,5'-oligoadenylate synthase (OAS1) and ubiquitin-like IFN-stimulated gene 15-kDa protein (ISG15) expression. On the other hand, at the time of luteolysis, LPA abrogated tumor necrosis factor (TNF)α and interferon (IFN)γ as well as nitric oxide (NO) - induced inhibition of P4 synthesis in the bovine CL. LPA exerted this luteoprotective role during the CL luteolysis via the modulation of the cytokines and NO initiated apoptosis. In the bovine ovarian follicle, LPA stimulated estradiol (E_2) production and FSH action in granulosa cells *via* increased expression of the FSHR and 17β-HSD genes, which in turn accounted for the participation of LPA in ovarian follicle growth and differentiation. On the other hand, during oocyte maturation *in vitro*, the

supplementation of the oocyte medium with LPA stimulated the expression of genes related to oocyte quality (*FST, GDF9, CTSs*) in oocytes and cumulus cells and also related to developmental competence (*OCT4, SOX2, IGF2R*) of oocytes and subsequent blastocysts. Moreover, LPA promoted an antiapoptotic balance in transcription of apoptosis-related genes (*BAX* and *BCL2*) in oocytes and blastocysts. These effects, although not translated into enhanced blastocyst *in vitro* development, may be relevant for *in vivo* embryo survival. Moreover, LPA directed glucose metabolism toward glycolytic pathway. LPA increased glucose uptake by cumulus-oocyte complexes *via* augmentation of *GLUT1* expression in cumulus cells and stimulating lactate production *via* enhancement of *PFKP* expression in cumulus cells. LPA did not affect cumulus expansion as visually assessed, however, it stimulated upstream genes of cumulus expansion cascade, *AREG* and *EREG*. We also demonstrated that LPA is an early bovine embryonic autocrine/paracrine signaling mediator, and its action during early embryo-maternal interactions led to better embryonic survival.
In conclusion, the obtained data indicate that LPA can play the supportive role in embryo-maternal interactions in the cow.

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Early embryo-maternal communication in natural pregnancy and after embryo-transfer in mice

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Preimplantation dialogue

There is a growing body of evidence that the suggestion made by Fujiwara [1] about the early embryo recognition by the maternal tract and maternal immune system, is genuine. Several observations support this idea of alteration taking place in oviduct and uterus in the presence of embryo [2; 3]. The results of the present study reinforce the hypothesis that the embryo elicits a local response in the uterine horns. It modulates maternal response by altering the activity of signal transduction pathways in the endometrial tissue.

In mouse, short period of preparation of the uterus for implantation, which culminates in the opening of the implantation window, leads to changes in the metabolism of uterine epithelial and stromal cells. This process is controlled by ovarian production of steroids, but it seems that embryo development and uterus remodeling are independent processes. It is known that embryos are able to develop till blastocyst stage *in vitro* but it is possible to mimic decidualization without the presence of conceptus.

Thus, it is believed that communication between the mother and the embryo is one of the most important steps in maintenance of pregnancy. During early stages of gestation (preimplantation period), trophoblast cells don't have direct cell to cell contact with the uterine layer. Embryo-maternal dialogue is created by auto-, endo- and paracrine interactions: secretion of steroid hormones and other signaling molecules.

Such bidirectional information is still poorly recognized, which might be the reason of lower reproductive performance of females after treatment with assisted reproductive technologies (ART). Disturbed embryo-maternal communication in a period directly preceding implantation may underlie pregnancy development, birth rate and newborns' health and conditions [4].

Study design

Embryo- maternal communication is considered to manifest primary by short signals – locally in the uterus. To track those signals we investigated gene expression level of nine chosen signaling pathways: WNT, hedgehog, Notch, NfκB, Jak/Stat, LDL, mitogenic, p53 and TGFβ pathway. The research was divided into two experiments. The first experiment was conducted on group of mice in preimplantation pregnancy in comparison to pseudopregnant females, to answer the question about signaling during natural gestation. The second experiment was based on embryo-transfer technique, to investigate how embryos with different biological quality affect local embryo-maternal communication. Investigation of gene expression of 9 signaling pathways, in uterine horns with confirmed presence of embryos and in control ones, was performed by real-time PCR reaction with the use of RT^2 PCR Array (SABioscience, USA) and ABI 7900 Thermal Cycler System. Custom made, self-selected gene panel with nine signaling pathways was used (Table 1), covering in total investigation of 84 gene expression levels.

Table 1. List of investigated signaling pathways and their representative genes.

WNT PATHWAY *Birc5 Ccnd1 Cdh1 Fgf4 Jun Lef1 Myc Pparg Tcf7 Vegfa Wisp1 Axin2 Ccnd2*
HEDGEHOG PATHWAY *Bcl2 Bmp2 Bmp4 Ptch1 Foxa2 Hhip Wnt1 Wnt2 Wnt3 Wnt5a Wnt6*
JAK/STAT PATHWAY *Irf1 Ccnd1 Sosc3 Gata3 Il4ra Mmp10 Nos2 Cxcl9*
MITOGENIC PATHWAY *Egr1 Fos Jun Nab2 Egrf1 Map3k1 Map3k2 Map2k1 Map2k2 Mapk1 Mapk3 Atf2 Trp53*
NOTCH PATHWAY *Hes1 Hes5 Hey2 Hey1 Id1 Jag1 Notch1 Pparg Ccnd1 Cd44 Il2ra*
LDL PATHWAY *Ccl2 Csf2 Sele Selp Vcam1*
P53 PATHWAY *Bax Cdkn1a Egfr Fas Gadd45a Pcna Rb1 Igfbp3 Mdm2 Tnf Trp53*
TGF-B PATHWAY *Ckn1a Cdkn1b Cdkn2a Cdkn2b Atf4 Myc Sox4 Id1 Id2 Stat1*
NFKB *Cxcl1 Icam1 Nfkbia Nos2 Vcam1 Stat1 Myd88 Birc3 Il2a*
OTHER SINGLE GENES INCLUDED *Hoxa10 Ptgs2 Lif*

Results

During the first experiment we have compared two groups of outbred CD-1 mice: in pseudopregnancy and in pregnancy at 3,5 day after mating, before implantation of the embryo. Results revealed down-regulation of majority of tested genes (50 out of 84 genes;

60%). Among 8 statistically relevant genes, 6 were down-regulated (Atf2, Birc3 Ccl2, Id2, Map3k ,Vcam1) and two up-regulated (Myd88, Fos) (Fig.1). Significantly altered genes belonged mainly to mitogenic (early-responsive genes) and NfκB pathway.

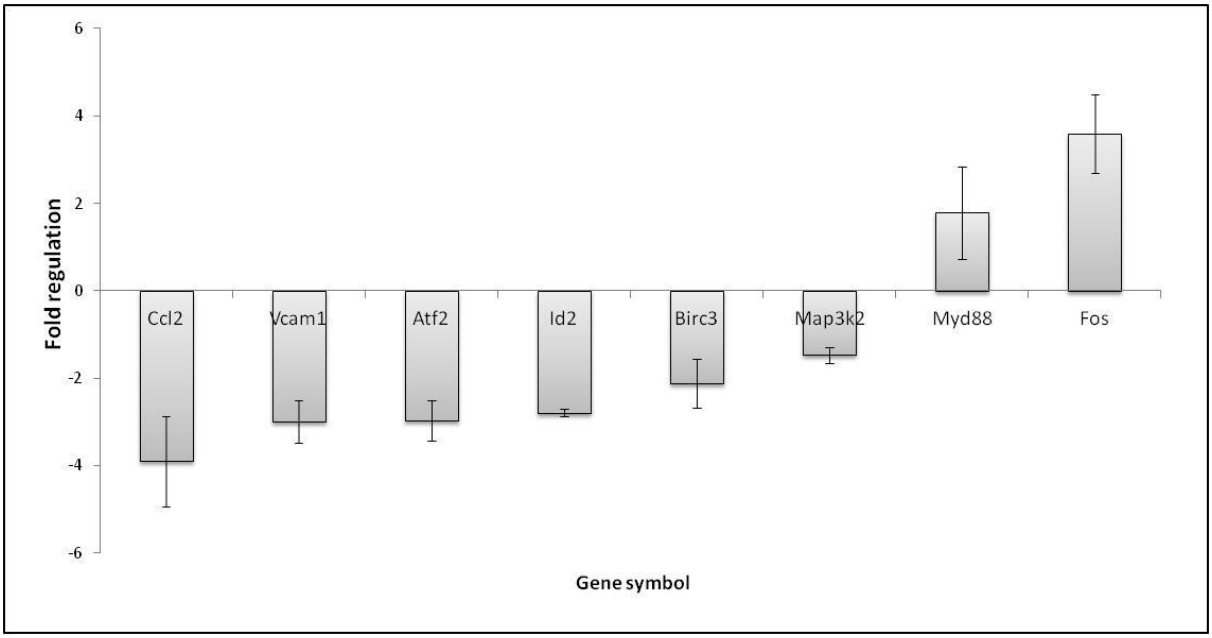

Figure 1. Relative level of significantly different expressed genes in uterus of pregnant (3,5 day post mating) comparing to pseudopregnant CD-1 mice, normalized to β-actin as a reference gene. p <0,05; Student t-test, Mann- Whitney test.

In the second experiment we employed mouse non-surgical embryo transfer (ET) technique to investigate the differences in mother- embryo signaling between normal embryos and embryos with reduced biological potential induced by *ex vivo* TNFα treatment. 1,5 dpc embryos derived from donors were cultured for 24 h *in vitro* without or in the presence of TNFα and transferred to hormonally synchronized, progesterone treated recipients. TNFα treated embryos showed increased number of apoptotic blastomers, however they were able to continue their development till blastocyst stage *in vitro*. Uterine gene expression after embryo transfer was compared to control group after intrauterine transfer of culture medium with or without TNFα. Results demonstrate that ET procedures, in contrast to normal pregnancy, influence regulation of majority of examined signaling pathways.

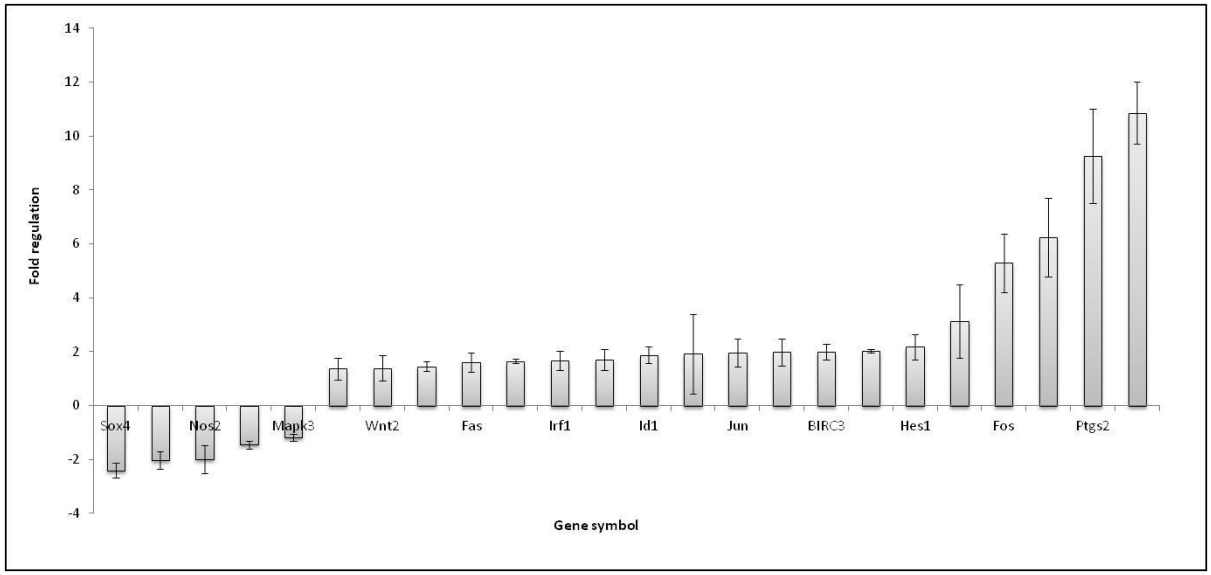

Figure 2. Relative level of significantly different expressed genes in uteri of CD-1 mice after non-surgical transfer of normal embryos to murine uteri comparing to group of mice after intra-uterine transfer of culture medium normalized to β-actin as reference gene. $p<0,05$; Student t-test, Mann- Whitney test.

Moreover, gene expression in murine preimplantation uterus differs depending on the quality of transplanted embryo. ET of biologically competent embryos alters 24 genes expression, among which 79% were up-regulated. Whilst transfer of TNFα-treated embryos down-regulates expression of 41 genes, while only one gene (Fos) displaying increased expression. Observed changes include involvement of crucial to implantation signal transduction pathways: NfκB, TGFβ, WNT and Hedgehog as well as Hoxa10 and Ptgs2 genes additionally included into the study.

Conclusion

The results of this study revealed that during natural pregnancy maternal response to embryo presence at preimplantation stage is discreet and subtle, with only 9% of significantly regulated genes within examined signal transduction pathways. Majority of them belonging to NfκB and mitogenic pathway. Another conclusion that emerges from the second experiment is differential gene expression depending on the quality of transplanted embryo. Those differences refer to the number of altered genes (24 and 42 in mice after transfer of normal and biologically impaired morulas) and direction of regulation ranging from up-regulation in ET and down-regulation after ET with TNFα- treated embryos. Our results indicate that different external conditions of embryo development profoundly influence eliciting of signal transduction pathways in the uterus of pregnant female crucial for pregnancy maintenance and development.

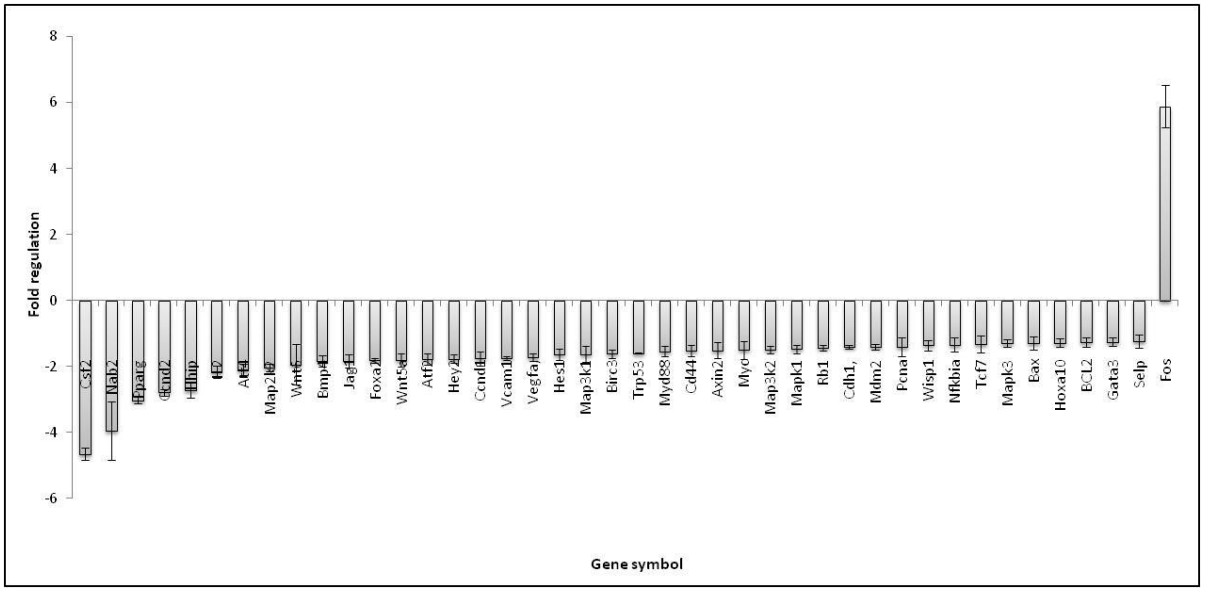

Figure 3. Relative level of significantly different expressed genes in uteri of CD1 mice after transfer of *ex vivo* TNFα –treated embryos compared to group of mice after intra-uterine transfer of culture medium with TNF-α, normalized to β-actin as reference gene $p<0,05$; Student t-test, Mann-Whitney test.

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Ovarian and placental molecular mechanisms responsible for pregnancy maintenance and prepartal luteolysis in cats

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Considering the high importance of the domestic cat as a model species for studying the reproductive biology of wild cats, and its role as one of the most important pets, more effort needs to be put into a better understanding of the feline reproductive physiology.

The length of the luteal phase in pregnant queens, depicted in elevated progesterone (P_4) levels, is assumed to be partially driven by luteotrophic and luteolytic factors of placental origin. The higher P_4 values reported for pregnant queens may result from P_4 supplementation by placental tissue, as hypothesized previously. Almost identical intraluteal expression profiles of steroidogenic enzymes, as well as intraluteal steroid contents, strengthened this hypothesis. We provided data, showing that *corpora lutea* (CLs) of the same histomorphological stage are characterized by identical steroidogenic capacities independently of an on-going pregnancy [1]. Thus, the feline placenta was assumed to be an additional source of P_4. Both StAR and 3βHSD were immunolocalized in the placenta and CLs within each luteal phase. The successful extraction of P_4 from the placenta further supports its role as an additional source of P_4 in the cat. Placental P_4 concentrations seem to be dependent on the gestational age [2]. The question, whether P_4 originating from the placenta alone can maintain pregnancy in domestic cats ovariectomized after 45 days of gestation, is still open.

In most species, the demise of CLs is connected to decreasing levels of serum P_4 and increasing levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$); most likely as a prerequisite for prepartal luteolysis. However, the capacity of feline CLs to provide $PGF_{2\alpha}$ was limited during the pregnant and non-pregnant luteal phases and mRNA levels of $PGF_{2\alpha}$ synthase (*PGFS*) were relatively low [3]. Thus, the intraluteal production of $PGF_{2\alpha}$ is presumably not, or only to a small extent, involved in the initiation of prepartal and cyclic luteolysis. Therefore, the question, whether the feline placenta is capable of synthesizing prostaglandin $PGF_{2\alpha}$ during the course of pregnancy, was addressed. The placental PGFS, which is classified as an aldo-keto reductase 1C3 (AKR1C3), was analyzed at the mRNA and protein level throughout

pregnancy and before parturition. Blood plasma was collected for immunoassaying P_4 and the $PGF_{2\alpha}$ inactive metabolite 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM). Both protein and transcripts for PGFS (AKR1C3) were strongly up-regulated soon after implantation, which takes place on days 12–14 after ovulation in the cat, and then gradually declined towards the end of pregnancy. The PGFS protein was elevated, particularly at 2.5–3 weeks of pregnancy compared to 7–8 and 8.5–9 weeks. As it was expected, transcripts for *PTGS2* were solely up-regulated in placentas from queens close to term. Staining for PTGS2 showed distinct positive signals in placentas obtained during the last week before labor, particularly in the strongly invading trophoblast surrounding blood vessels, but also in decidual cells. Shortly after implantation, signals for PGFS were localized in the trophoblast cells. Near term, PGFS staining was seen mainly in decidual cells. Both placental $PGF_{2\alpha}$ and plasma PGFM were elevated towards the end of pregnancy. The content of $PGF_{2\alpha}$ in extracted placentas mirrored the PGFM level in plasma of pregnant females [4]. These findings led to the conclusion that even if PGFS (AKR1C3) was low just before term, with accompanying high placental $PGF_{2\alpha}$ concentrations and high levels of circulating PGFM, an enzyme responsible for prostaglandin metabolism, namely 15α-prostaglandin dehydrogenase (PGDH), might be involved in $PGF_{2\alpha}$ bioavailability. In addition, an involvement of aldo-keto reductase B5 (AKR1B5) in $PGF_{2\alpha}$ synthesis might also be considered, and especially the fact that AKR1B5 might be able to inactivate P_4 locally, despite elevated P_4 levels in the maternal blood.

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Expression of equine major histocompatibility complex class I during pregnancy, parturition and fetal membrane retention

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We hypothesized that the fetus will down-regulate MHC I expression to avoid maternal recognition during pregnancy, but will up-regulate MHC I expression during parturition so that the mother's immune system will reject fetal cells, facilitating release of fetal membranes. Moreover, we further hypothesized that lower expression of fetal MHC I during parturition will be associated with fetal membrane retention. To test these hypotheses, placenta samples were collected from 16 pregnant heavy draft mares in a slaughterhouse. The pregnancies ranged from 1 to 8 months old. We also took biopsies from mares during parturition, immediately after foal delivery (n=33) (gestation length 320-350 days). These mares were then monitored for the time of fetal membrane expulsion. Fetal membrane retention (FMR) was defined as retention of the membranes for 3 hours. There were 13 FMR mares and 20 control mares. As a further control, endometrial biopsies were taken from 5 mares in anestrus. For qPCR primers were designed to detect all the best-known equine MHC I genes (5 genes: NM_001082505.1, X79890.1, X79891.1, X79892.1, AY225157.1 mut) [1]. To detect MHC I protein, mouse anti-horse MHC I monomorphic antibodies were used (MCA1086GA Serotec and ab23491 Abcam) according to procedures described by Rapacz-Leonard et al. [2]. To test normality, data were analyzed by the D'Agostino and Pearson omnibus normality test. To test significance, Student's T-test or ANOVA were run using GraphPad Prism.

During pregnancy, MHC I mRNA was strongly up-regulated in the endometrium in the first month of pregnancy (at least 4.6 times higher than in any other month) (P=0.0008). After the first month of pregnancy, MHC I mRNA expression was down-regulated in the endometrium, but it was higher than during months 6–8 of pregnancy. In the allantochorion, MHC I mRNA expression was constant throughout pregnancy. With respect to MHC I protein, its levels were measured during months 3–8 of pregnancy and were barely detectable.

To confirm the detection of MHC I, equine lymph node was used as a positive control. MHC I levels in the lymph node were at least 41 times higher than in placental samples (P=0.03).

During parturition, MHC I mRNA expression in the allantochorion was higher than during months 6–8 of pregnancy, but its expression in the endometrium was the same as during those months of pregnancy. MHC I mRNA expression was at least 2.5 times higher in the allantochorion than in the endometrium. Moreover, MHC I mRNA expression in the allantochorion was 3 times higher in mares that retained fetal membranes than in mares that delivered fetal membranes physiologically (P=0.008). MHC I mRNA expression in the endometrium was very low when compared to the endometrium during anestrus (P=0.003). With respect to MHC I protein during parturition, its levels in the endometrium were 3.2 times higher than in the allantochorion (P=0.0005). Moreover, MHC I protein levels in the endometrium were 1.7 times higher in mares that delivered fetal membranes physiologically than in mares with fetal membrane retention (P=0.0079). MHC I protein levels in the allantochorion were 1.1 times higher in mares with fetal membrane retention than in mares with physiological delivery of fetal membranes (P=0.0159).

In conclusion, maternal and fetal MHC I protein levels were very low during months 3–8 of pregnancy. Because MHC I mRNA was expressed during these months, this might indicate–post-translational and/or transcriptional regulation. MHC I mRNA expression was up-regulated at parturition, which might indicate that fetus prepares the mother's immune system for fetal membranes recognition and rejection. However, because protein levels were determined with different systems, this needs further study. Interestingly, MHC I mRNA and protein expression were higher in the allantochorion of mares with fetal membrane retention than in mares that delivered the membranes physiologically, whereas MHC I protein levels were lower in the endometrium of mares that retained fetal membranes.

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Brain mechanism underlying ovulation in mammals

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Mammals can be divided into two groups based on their ovulatory processes: one is the spontaneous ovulator and the other is the reflex ovulator. In the spontaneous ovulator, including mice, rats, pigs, goats and cows, follicular development and ovulation occur in a cyclic manner. High levels of estrogen produced by matured follicles induce gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) surge and then ovulation through estrogen-positive feedback action. On the other hand, in the reflex ovulator, including rabbits, ferrets and musk shrews, follicular development occurs spontaneously, whereas ovulation does not occur without mating. They ovulate only when they receive the mating stimulus by males.

In 1971, GnRH was identified as a molecule to induce gonadotropin release from the gonadotroph of the anterior pituitary. GnRH cells are mainly located in the preoptic area (POA) of non-primate mammals and in the medial basal hypothalamus of primates. GnRH fibers project to the median eminence and GnRH is carried to the pituitary through the hypophyseal portal blood. It is unlikely that GnRH cells directly receive estrogen-positive feedback action because no estrogen receptor alpha (ER alpha) expression was found in GnRH cells. Thereby, the mechanism of GnRH regulation *via* estrogen signaling has been a mystery for more than 30 years. Kisspeptin, a newly found neuropeptide encoded by *Kiss1* gene in 2001, has broken through some of this mystery. ER alpha is expressed in kisspeptin neurons and kisspeptin receptor, *Gpr54,* is expressed in GnRH cells. In the recent decade, it has been clarified that kisspeptin-GPR54 signaling plays a crucial role in initiating secretion of GnRH/LH in mammals, including both spontaneous and reflex ovulators.

Kisspeptin neurons are located in two regions of hypothalamus: the anteroventral periventricular nucleus (AVPV)/POA and the arcuate nucleus. AVPV/POA kisspeptin neurons are considered to be responsible for estrogen-positive feedback action. *Kiss1* mRNA expression in the AVPV was stimulated by estrogen in ovariectomized (OVX) rats [1]. A central infusion of rat anti-kisspeptin antibody blocked spontaneous and estrogen-induced LH surge in female rats [1, 2]. Moreover, in goats and monkeys, the estrogen-induced LH surge was accompanied by an activation of POA kisspeptin neurons [3, 4]. Thus, in the spontaneous

ovulator, high levels of estrogen activate AVPV/POA kisspeptin neurons to induce GnRH/LH surge and then ovulation (Fig. 1).

Kisspeptin-GPR54 signaling also plays a significant role in the reflex ovulator, such as musk shrews (*Suncus murinus*). In female musk shrews, POA kisspeptin neurons were activated by the mating stimulus [5], and an administration of kisspeptin or GPR54 agonists mimicked the mating stimulus to induce ovulation *via* GnRH release [5]. In other reflex ovulators, such as rabbits and ferrets, it has been reported that the administration of estradiol did not induce LH surge in female animals [6, 7]. Taken together, in the reflex ovulator, the mating stimulus but not estrogen signaling activates POA kisspeptin neurons to induce GnRH/LH surge and then ovulation (Fig. 1).

This presentation reviews the brain mechanism underlying spontaneous and reflex ovulation, focusing on kisspeptin-GPR54 signaling.

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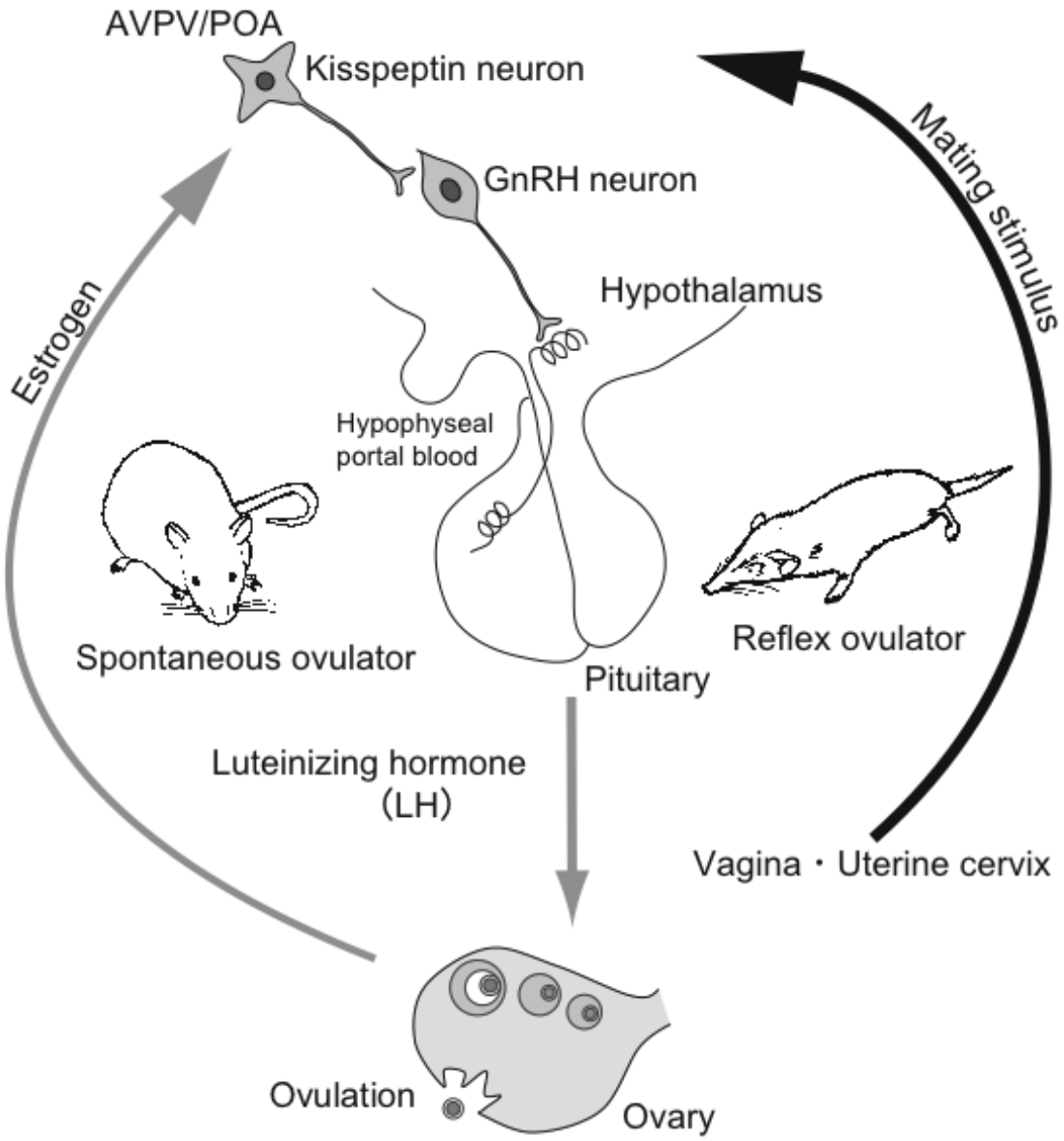

Figure 1. Scheme of the ovulatory mechanism in mammals. In the spontaneous ovulator, estrogen signaling activates AVPV/POA kisspeptin neurons to induce GnRH/LH surge and then ovulation. In the reflex ovulator, the mating stimulus activates POA kisspeptin neurons to induce ovulation *via* GnRH release.

Electrophysiological Technique for Monitoring the Hypothalamic Mechanism Regulating Pulsatile GnRH Release in Goats

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Reproductive processes in female mammals, such as follicular development in the ovary, ovulation of dominant follicles, lactation and sexual/maternal behaviors, are under control of the brain. The release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is the top of neuroendocrine control hierarchy of reproductive functions and the pattern of GnRH release is a key determinant regulating gonadal activities. The basal release of GnRH is pulsatile and each GnRH pulse is associated with an increase in synchronized electrical activity (multiple unit activity; MUA) in the mediobasal hypothalamus. The increase in the MUA is called "MUA volley" and this is thought to reflect the rhythmic oscillations in the activity of the neuronal network, the GnRH pulse generator, which drives pulsatile GnRH secretion [1]. Indeed, temporal correlations between MUA volleys recorded in the mediobasal hypothalamus and luteinizing hormone (LH) pulses in peripheral circulation have been shown in monkeys [2], goats [3] and rats [4]. The cellular source of this ultradian rhythm in GnRH release has not been fully elucidated; although many research groups in this field have tried to unravel the mechanism.

Recent studies in the field of reproductive neuroendocrinology reveal that a subset of neurons identified in the hypothalamic arcuate nucleus (ARC) coexpresses three neuropeptides, kisspeptin, neurokinin B (NKB), and dynorphin; each of three peptides has been shown to play a critical role in the central control of reproduction [5]. Growing evidence suggests that these neurons, KNDy neurons named after initial letters of three peptides, are conserved across a range of species from rodents to humans and play a key role in the regulation of pulsatile GnRH release [6]. To determine whether the KNDy neuron signaling could be responsible for producing pulsatile GnRH secretion, we recorded the MUA in the close vicinity of KNDy neurons in the posterior ARC, where the majority of KNDy cells are located in goats. Goats were implanted with an array of bilateral recording electrodes aimed at the posterior portion of the ARC according to the stereotaxic procedure. The electrodes consisted of six Teflon-insulated platinum-iridium wires (75 micrometer in diameter) in each

side. The method for recording MUA has been described elsewhere [7]. Rhythmic volleys of the MUA were found to be accompanied by LH pulses with regular intervals (Figure 1).

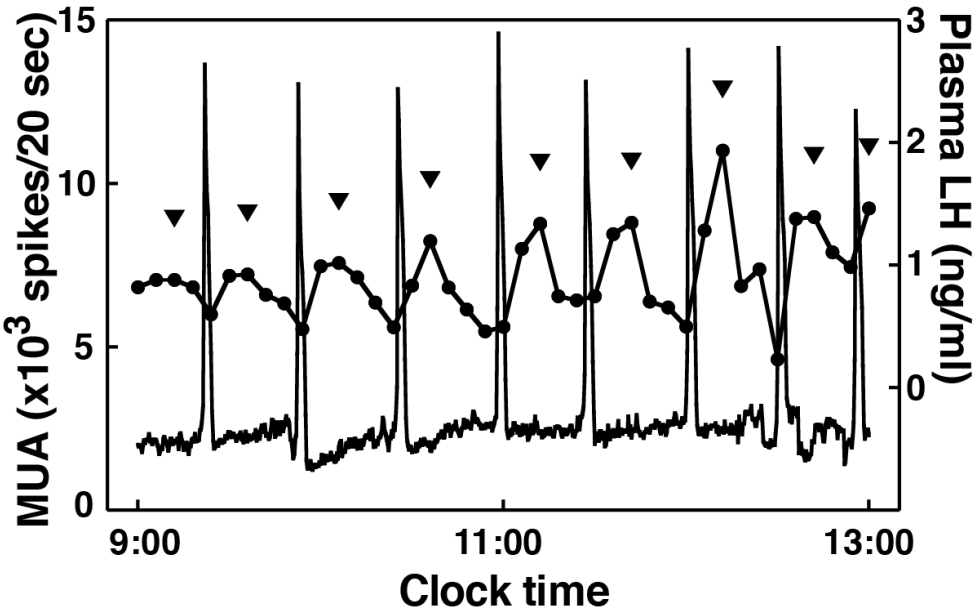

Figure 1.A representative profile of the MUA (solid line) and plasma LH concentrations (solid circles) in an ovariectomized goat. An array of bilateral recording electrodes was implanted aiming at the posterior portion of the ARC of the hypothalamus, where the majority of KNDy cells are located. Arrowheads represent the peaks of LH pulses identified by the PULSAR computer program. Note that each LH pulse follows the corresponding MUA volleys throughout the sampling period.

Exogenous administration of NKB and dynorphin stimulated and suppressed, respectively, regular occurrence of rhythmic volleys of the MUA [8]. In contrast, administration of kisspeptin peptide stimulated a sustained increase in LH secretion, without influencing intervals of the MUA volleys [9], suggesting that the GnRH pulse generator, as reflected by the MUA, originated from outside of the network of GnRH neurons. Rhythmic electrophysiological activity could plausibly reflect the pacemaker activity of KNDy neurons, whose projections reach the median eminence where GnRH fibers project. These observations suggest that the KNDy neurons in the ARC may be the intrinsic source of the GnRH pulse generator.

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Effect of endotoxin on ovarian follicle growth regulation in catle

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In dairy cattle, uterine bacterial contamination is dynamic, with regular contamination, clearance of bacteria, and spontaneous recontamination during the first few weeks after parturition. Ovarian function is perturbed in cattle with greater uterine bacterial contamination after parturition. The endotoxin lipopolysaccharide (LPS), which is a component of gram-negative bacterial walls, is increased in the plasma of cows with uterine infection. A recent study has reported that there is a relationship between uterine infection, endotoxin production and resumption of postpartum ovarian activity [1].

Effect of LPS on ovarian follicular function: In our study, LPS was detected in the follicular fluid of large follicles (>8 mm in diameter) from the bovine ovary. In follicles with a high level of LPS, the concentration of E2 was lower and that of P4 was higher when compared to those with a low level of LPS. In addition, we observed the drastic changes in transcription levels of steroidogenesis-related genes. These results indicate that LPS present in follicular fluid may inhibit follicular activity in bovine ovary. In our study, the mRNA expression of CYP17 in theca cells and P450arom in granulosa cells was lower in follicles with a high level of LPS when compared to follicles with a low level of LPS.

Effect of LPS on granulosa cell function: We examined the effect of LPS on E2 production in granulosa cells isolated from small and large follicles. LPS disturbed the production of E2 but not P4 in FSH-treated granulosa cells from small and large follicles. Our data indicated that LPS induced the expression of its receptor, TLR4, CD14 and MD2, in granulosa cells treated with FSH. Their receptors induced by LPS may contribute to suppression of E2 production in granulosa cells from small follicles. LPS stimulation was shown to upregulate CD14 and TLR4 expression and trigger close physical proximity between CD14 and TLR4 in monocytes [2]. Our data demonstrated that 10 μg/ml of LPS stimulated transcription of *TLR4, CD14* and *MD2* genes in FSH-treated granulosa cells from small follicles. In contrast, LPS did not affect the expressions of *TLR4, MD2 and CD14* mRNA in granulosa cells from large follicles. These results suggest that small follicles have a higher sensitivity to LPS than large follicles in bovine ovaries.

Effect of LPS on theca cell function: We investigated the effect of LPS on steroid production in bovine theca cells at different stages of follicular development under LH conditions, E2 conditions, or both. LPS suppressed P4 and A4 production with downregulation of *StAR* and *CYP17* mRNA expression when theca cells were stimulated with LH. By contrast, LPS did not affect P4 or A4 production when theca cells were stimulated with E2. Bovine granulosa cells reportedly express TLR4, which recognizes LPS, and LPS suppresses E2 production in granulosa cells of bovine follicles [3; 4]. Similar to studies of bovine granulosa cells, our observation revealed that bovine theca cells expressed *TLR4*, *CD14*, and *MD2* mRNAs, which constitute the specific receptor for LPS [5], suggesting that bovine theca cells are capable of responding to LPS. Moreover, LPS suppressed the production of P4 and A4 in LH-stimulated theca cells, downregulating the transcription of steroidogenic enzymes *StAR* and *CYP17*. These findings indicate that LPS can act locally on bovine theca cells and suppress the steroidogenic function of theca cells as well as granulosa cells.

Figure 1 Follicular development

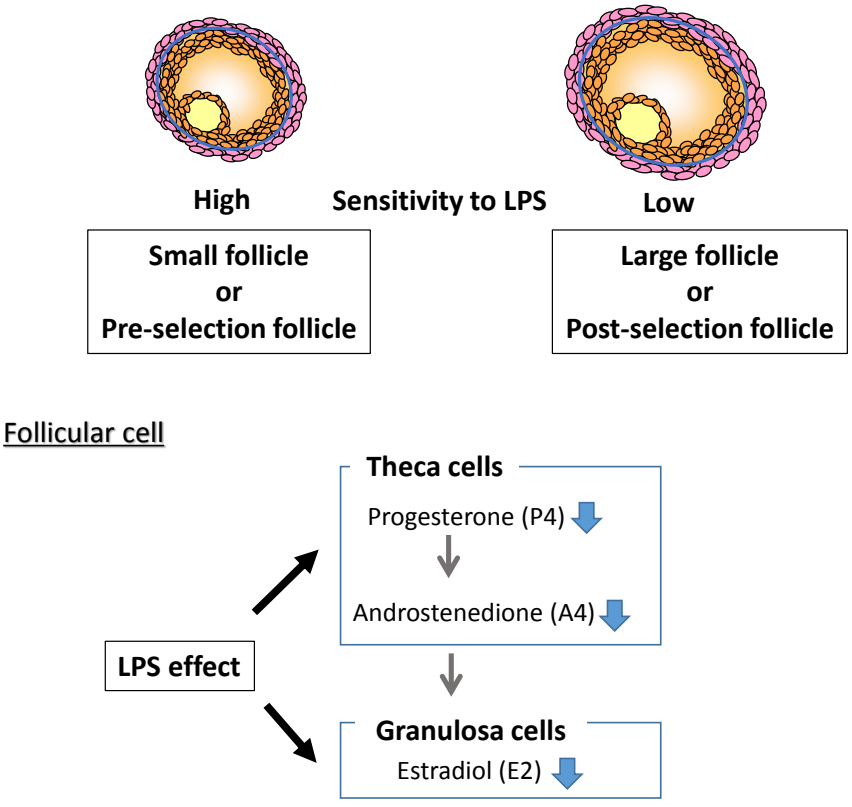

Figure 1. Effect of LPS on follicular development and follicular cell function in bovine ovary. During follicular development, small follicles have a higher sensitivity to LPS than large follicles in bovine ovaries. In follicular cell function, LPS disturbed the steroid production in granulosa cell and theca cell regardless of follicular growth.

Conclusion: We observed that in follicles with high level of LPS in follicular fluid, the follicular E2 concentration was lower compared to that in follicles with low level of LPS [6]. In those follicles with high level of LPS, mRNA expression of CYP17 in theca cells and P450aromatase in granulosa cells was lower. These findings indicate that LPS in follicular fluid influenced the steroid production in follicles. In addition to these observations *in vivo*, *in vitro* study has shown that LPS suppressed steroid production of granulosa cells [4] and theca cells [7]. This biphasic inhibitory effect of LPS might cause ovarian dysfunction and subsequent impaired fertility in cows with postpartum uterine infection.

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Genomic portrait of ovarian follicle growth regulation in cattle

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It is known that ovarian follicle development is controlled by endocrine hormones and intraovarian regulators, but the cellular and molecular regulation of follicle fate (proliferation or atresia) are still not fully understood. Sequencing of the bovine genome advanced research in many areas including studies on how gene expression in ovarian cells affects follicular growth. Research demonstrated that gene expression coordinates endocrine, intraovarian and other factors promoting growth of dominant follicle or regression of subordinate follicles [4, 8]. As follicle development progresses, levels of mRNA expression for markers of follicle maturation: *LHR* and *CYP19,* are greater in dominant compared to subordinate follicles [2, 4, 8]. Also, growth of dominant follicles is associated with increased expression of survival genes (i.e. *DICE-1, MCL-1*), while levels of genes linked to apoptosis (i.e. *COX-1*, *TNF-α*, *p58*, *Apaf-1*) are elevated in subordinate follicles [2]. Furthermore, expression of mRNA for *IGF-I* and *IGF-II* also increases in dominant follicles [9], while mRNA expression for *IGFBP-2, -5* decreases [7]. Expression of *INHBA*, *BMPR1A* and *BMPR2* (members of TGF-β family) is greater in large versus small follicles [3].

Gene expression is negatively regulated by small molecules called microRNAs (miRNAs), which cause mRNA degradation or translational inhibition through sequence-specific base pairing with target mRNAs, leading to post-transcriptional repression of gene expression [1]. To date, about 700 miRNAs have been identified in cattle. Some of them have been detected in bovine ovarian follicles.

Studies have shown that a set of miRNAs are differentially expressed between dominant compared to subordinate follicles and are involved in the regulation of known molecular pathways (e.g. Wnt, TGF-beta, ErbB, GnRH, MAPK, PI3K-Akt and oocyte meiosis signaling pathways) that determine the fate of follicular development [6, 10]. Among miRNAs that play an important role in follicle development is miR-582-5p, which has been proven to reduce *MCL-1* expression by binding to its 3'UTR site [5]. Expression of miR-582-5p was significantly higher in subordinate versus dominant follicles and pathway/target analysis verified that it regulates *MCL-1* in PI3K-Akt signaling [10]. Since increased expression of

MCL-1 is associated with growth of dominant follicles [2], miR-582-5p may be an important regulator of subordinate follicles undergoing atresia [10].

Understanding the interactions between mRNAs and miRNAs associated with dominant and subordinate follicles will provide a basis to unravel molecular factors involved in ovarian follicular development.

Acknowledgement

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Ovarian and endocrine function after hormonal induction of ovulation in seasonally anovular goats

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The primary goal of this series of experiments was to employ daily transrectal ultrasonography and hormone measurements to describe and compare ovarian activity and accompanying endocrine changes in seasonally anovular goats (July-August) receiving exogenous gonadotropins or buserelin acetate (gonadotropin-releasing hormone (GnRH) analogue), with or without the 14-day pre-treatment with intravaginal sponges containing 45 mg of fluorogestone acetate (FGA). The effects of a single i.m. dose of equine chorionic gonadotropin (eCG, 500 IU), human chorionic gonadotropin (hCG, 200 IU) or buserelin (0.01125 mg) on ovarian function, circulating concentrations of ovarian steroids and periovulatory secretion of luteinizing hormone were evaluated in ultrasonographically monitored animals. Each experiment utilized 12 goats of the Polish White breed that were randomly allocated to two equal groups (FGA-primed [TRT] vs. untreated controls [CTR]) and examined for a total of 42 days, with induced ovulations occurring 21 days into the observation period.

In CTR goats, there were more follicles emerging in the ovulatory follicular wave (defined as a group of antral follicles attaining ostensibly ovulatory sizes before regression or ovulation) that grew to ≥5 mm in diameter but fewer ($p<0.05$) ovulations following the administration of eCG, hCG or GnRH compared with the TRT goats. Within the FGA-primed anestrous goats, the greatest ($p<0.05$) number of growing antral follicles in the ovulatory wave and the highest ($p<0.01$) ovulation rate were seen in eCG-stimulated animals. As in an earlier study by Lassale et al. [4], all ovulating follicles in animals pre-treated with FGA originated from only one wave. In spite of those differences, there were no effects of FGA treatment on the number of follicular waves observed during the entire period the FGA-soaked sponges were left in place, which is in agreement with previous studies in FGA-primed cyclic goats [5] and in anestrous ewes receiving Veramix® sponges (medroxyprogesterone acetate, 60 mg; [1]). Following the period of FGA pre-treatment and ovulations, the TRT group goats produced more follicles ≥4 mm medium-sized, ($p<0.05$) and numerically more ≥5-mm follicles (large) than their control counterparts. Specifically, the numbers of medium and large ovarian

follicles in the first wave after ovulation was reduced compared with the ovulatory follicular wave in the CTR anestrous goats, most likely due to reduced ovarian sensitivity to gonadotropic stimulation after progestin exposure and post-ovulation [2].

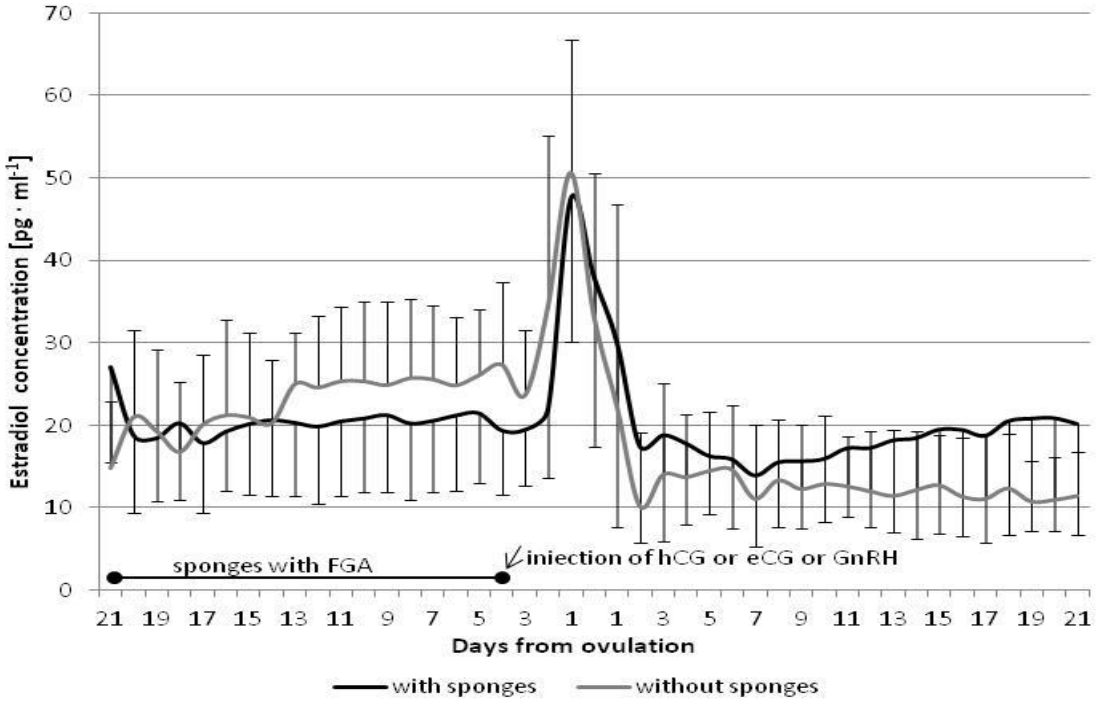

Figure 1. Mean (+ or -SD) daily concentrations of estradiol in anestrous goats with or without fluorogestone acetate (FGA) sponge pre-treatment during the entire 42-day study period (Day 0=ovulation).

An increased number of medium and large antral follicles in the first post-ovulatory wave of the TRT goats was accompanied by elevated circulating concentrations of estradiol compared with the CTR group [Fig. 1]. A similar post-ovulatory rise in follicular estradiol secretion was observed by Khalid et al. [3] in seasonally anestrous ewes pre-treated with progesterone. This increased estrogenicity of antral follicles was seemingly a result of an initial stimulation with exogenous gonadotropins and endocrine environment exerting a modulatory effect on the population of ovarian follicles growing during the period of corpus luteum (CL) formation; all animals that had received FGA sponges ovulated and had fully functional corpora lutea whereas none of the CTR goats induced to ovulate with eCG, hCG or GnRH after ovulation formed normal CL. Interestingly, the formation of corpora lutea that were subsequently detectable with transrectal ovarian ultrasonography for a period corresponding to the normal length of the luteal phase was observed in the CTR goats. Moreover, luteal glands in the CTR group appeared to exhibit similar echotextural charatertics to those seen in the TRT goats. However, serum progesterone concentrations were significantly depressed in the CTR goats post-ovulation regardless of the gonadotropin used to induce synchronous ovulations.

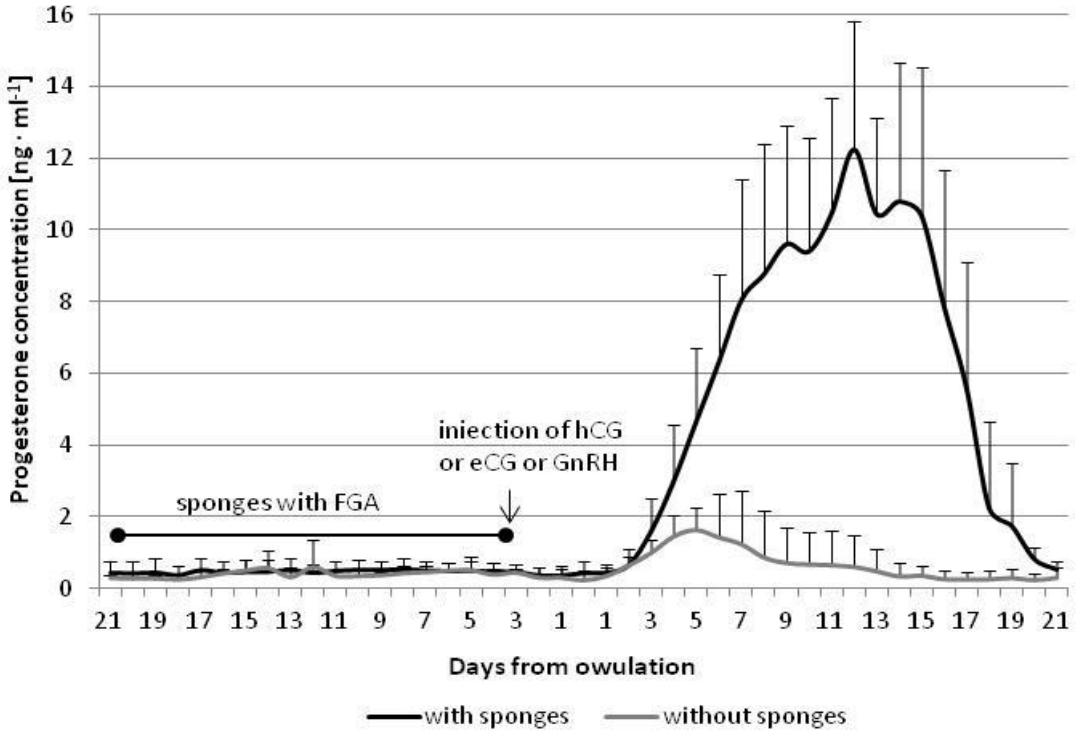

Figure 2. Mean (+ or -SD) daily concentrations of progesterone in anestrous goats with or without fluorogestone acetate (FGA) sponge pre-treatment during the entire 42-day study period (Day 0=ovulation).

During the first few days post-treatment, corpora lutea in CTR animals only synthesized small amounts of progesterone and at 8 days after ovulation, serum progesterone levels in this subset of goats declined to <1 ng/ml [Fig.2]. Collectively, these observations can be interpreted to suggest that progestin priming of seasonally anovular goats is a prerequisite for adequate development of granulosa cells and their steroidogenic capacity, which in turn leads to physiological progesterone biosynthesis by the resultant luteal cells [1; 2]. Lastly, persistent ovarian follicles were detected in goats after eCG and hCG treatments but not in the buserelin-treated animals.

In summary, the treatment of anestrous goats with FGA did not affect the number or kinetics of emerging follicular waves. In all experiments, control animals tended to have more ovarian antral follicles ≥5 mm that grew to larger maximum diameters than those in FGA-treated goats, but had fewer ($p<0.01$) ovulations compared with their FGA-primed counterparts. The FGA-treated goats had significantly lower circulating concentrations of estradiol compared

with controls post-treatment. All goats that had received FGA sponges before the treatment with eCG, hCG or GnRH analogue developed fully functional CL post-ovulation whereas all control animals only had inadequate CL secreting small amounts of progesterone. The present results clearly indicate the modulatory effects of a synthetic progestin on ovarian antral follicle development, estrogenicity and gonadropin responsiveness as well as on ensuing luteal function in anestrous goats induced to ovulate with exogenous gonadotropins or a GnRH analogue. The use of exogenous gonadtropins appeared to be associated with the prolonged antral follicular growth post-treatment.

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Closing remarks

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Japanese and Polish researchers, who are renown–pioneers in reproductive physiology, biotechnology and pathology, have met for the third time in the last ten years under our joint Seminar between Polish Academy of Sciences and the Japanese Society for promotion of Sciences. Still, the main goal of such event is to encourage ~~the~~ young scientists of both countries to establish further long-lasting collaborations, which are expected tp result in joint research projects.

In fact, since 2000 a number of joint research projects was established and realized, resulting in more than two hundred joint publications in the field of reproductive biology and pathology. We expect that also this Seminar will be as fruitful and in the near future young researchers from both nations will begin active collaborations and scientific exchange programmes, and this way to exchange information, leading to a sustainable progress reproductive biology.

The new directions of our future research were analyzed and defined and we can expect significant progresses in field of reproductive biology and pathology. Finally we hope that the Seminar should enable us to make at least the research in our filed more public and practical.

Here, I want to thank particularly Dr. Marta Siemieniuch, Dr Anna Szóstek and Karolina Łukasik for their hard work in organizing the current meeting. Organizing an international meeting is not an easy job. I want to also thank all our speakers and all of you for participating in the meeting.

I believe that in the soon future we can further increase our collaboration. We hope that all participants will return home stimulated and with new research ideas and new friendships.

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