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Molecular Veterinary Medicine

Programme Coordinator: **Prof. RNDr. MVDr. Petr Hořín, CSc.**

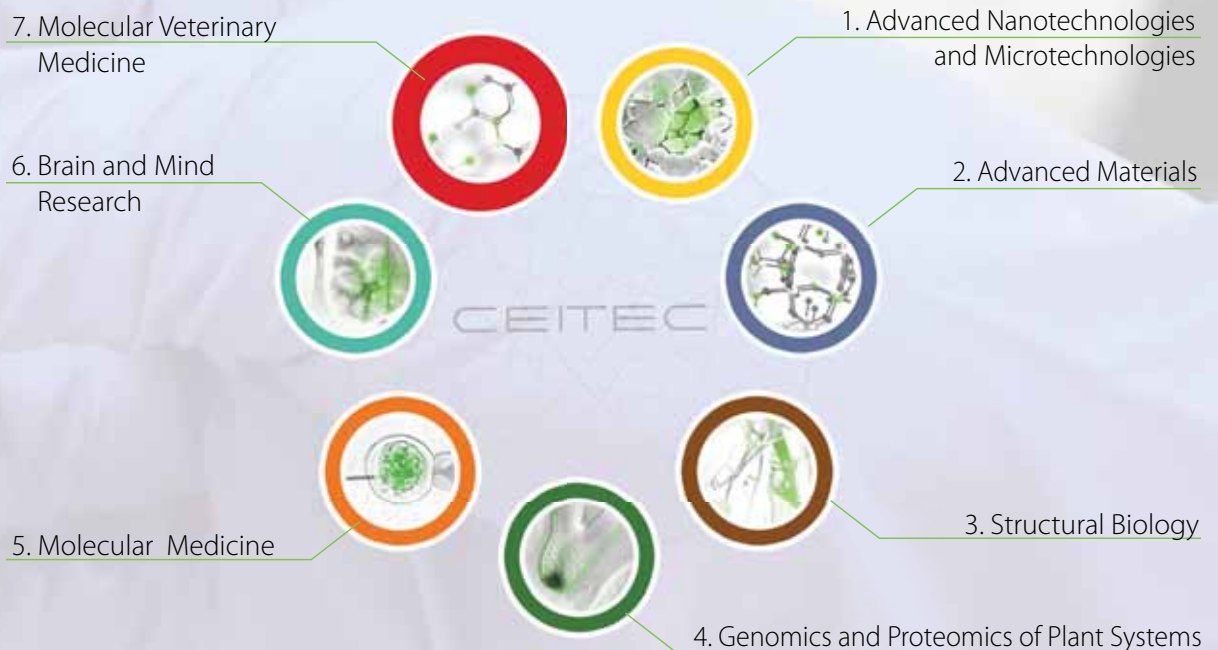


Characteristics of the Research Programme

Important biological processes and diseases will be studied in selected animal models. Complex mechanisms of resistance to infectious diseases and of mammalian reproduction will be analyzed by using various methodological approaches, including host and pathogen genomics and proteomics. Based on this knowledge, potential practical applications will be investigated, like prevention of circulation of important pathogens in the food chain and/or biotechnological potential of specific animal models of mammalian reproduction.

Overall Goal

Immunity and reproduction are the most important traits related to survival. Infectious diseases of animals have a significant economic impact and represent an environmental risk. This programme should promote the development of complex multidisciplinary approaches to study these basic biological processes and their potential applications in diagnostics, therapy, prevention and public health. Therefore, molecular and cellular mechanisms underlying host and pathogen interactions and mammalian reproduction will be studied. In the field of infectious diseases, pathogens causing important infections, including major foodborne pathogens and emerging pathogens at the human-domestic animal-wildlife interface will be studied in the context of host genetic mechanisms of disease. Possible applications, based on molecular techniques and on improvements in nanotechnologies and gene therapy, will be investigated. In the field of reproduction, chromosomes in somatic and germ-line cells, their evolution and role in reproduction will be analyzed. Mechanisms controlling acquisition of meiotic competence during oocyte growth and aging oocytes will be studied on animal models. Genomic, proteomic and bioinformatic approaches, cell culture, single cell techniques, live cell imaging, and biosensors will be used.



Research Directions

- Analysis of causes, mechanisms and spread of infectious diseases in domestic animals
- Analysis and prevention of circulation of zoonotic pathogens in the food chain
- Host genomics and genetics in infections and reproduction
- Animal models of mammalian reproduction and their biotechnological potential

Research Groups | Research Group Leaders

RG-7-1 | Molecular Virology | **Vladimír Celer**

RG-7-2 | Molecular Bacteriology | **Alois Čížek**

RG-7-3 | Parasitology | **Břetislav Koudela**

RG-7-4 | Food Safety | **Iva Steinhauserová**

RG-7-5 | Orthopaedics and Surgery | **Alois Nečas**

RG-7-6 | Animal Immunogenomics | **Petr Hořín**

RG-7-7 | Animal Cytogenomics | **Jiří Rubeš**

RG-7-8 | Mammalian Reproduction | **Martin Anger**

Work Packages

WP-7-1 | Analysis of the causes, mechanisms and spread of infectious diseases in domestic animals

WP-7-2 | Analysis and prevention of the circulation of zoonotic pathogens in the food chain

WP-7-3 | Host genetics and comparative immunogenomics

WP-7-4 | Comparative cytogenomics and the genetics of reproduction

WP-7-5 | Animal models of mammalian reproduction



1.1. Molecular Virology

Research Group Leader : Prof. MVDr. Vladimír Celer, DrSc.

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THEMATIC RESEARCH FOCUS

Research areas

- Porcine ssDNA viruses
- Porcine arteriviruses
- scFv antibodies
- Protein expression

Main objectives

- To obtain novel information on the causes, mechanisms and spreading of infectious diseases in domestic animals.

CONTENT OF RESEARCH

Analysis of the causes, mechanisms and spread of infectious diseases in domestic animals

Characterisation of virulence factors of viruses

The main objective is to elucidate the pathogenesis of some single stranded DNA viruses and arteriviruses in sows. Model viruses for this study will be porcine circovirus 2, porcine anelloviruses and porcine reproductive and respiratory syndrome virus. Sequencing of virus strains from different clinical conditions will be used to define ORFs, which could have the impact on virus virulence. Virus genomes will then be cloned into plasmid vectors to obtain infectious molecular clones as the conserved genetic material of these model viruses. Transcription of potential virulence factors will be subsequently modified by site directed mutagenesis and by siRNA molecules in vitro and in vivo models. In vitro model will be performed on tissue culture transfected with modified molecular clones and then analysed by quantitation of mRNA expression. For in vivo models infection of specific host animals will be used to analyse virulence of molecular clones.

Specific inhibition of virus replication by single-chain variable fragment mediated intracellular immunisation

The main objective is to evaluate the protective effect of intracellular immunisation mediated by single-chain variable fragment derived intrabodies against different viruses. We expect that intrabodies would inhibit afferent parts of the viral life cycle as well as replication of the virus by blocking virus maturation.

Porcine circovirus 2 and porcine arterivirus (PRRS) will be used as model viruses in this work package. Target virus proteins used for single-chain variable fragment selection will be expressed as recombinant proteins in *Escherichia coli* and *Pichia pastoris* expression systems. Recombinant single-chain variable fragment antibody fragments against these virus proteins (nonstructural proteins, nucleocapside) will be selected from appropriate phagemide library, e.g. Tomlinson, Griffin1. Selected single-chain variable fragment clones will be characterised by sequencing and determination of affinity constant. Gene coding variable fragments responsible for antigen binding will be cloned into plasmid vectors enabling expression of antibody fragments in different cell compartments. Then, transgenic cell lines will be established and challenged with corresponding virus strains. Inhibition of virus replication will be monitored by virus titration, antigen capture assay and immunofluorescence. To monitor at the genomic level (virus genome and messenger ribonucleic acid) the different phases of the virus replication cycle real-time PCR and reverse transcriptase - PCR combined with Southern blotting will be applied.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Molecular virology

Current research infrastructure

The research infrastructure of Molecular virology group allows the routine use of cell cultures, sequencing and cloning, expression and purification of viral antigens. The laboratory is equipped with ultracentrifuges with suitable rotors, equipment for the automated subtraction and evaluation of ELISA tests, equipment for SDS-PAGE, immunoblot and a thermal cyclic reactor for quantitative PCR. The laboratory is fully equipped for routine procedures associated with the cloning of virus genes into plasmid vectors and transformation or transfection of bacterial and eukaryotic cells, expression and the purification of recombinant proteins and other procedures

MAIN PROJECTS

- Porcine anelloviruses – prevalence, genotypization and pathogenesis (ME08108), Ministry of Education, Youth and Sports, 2008-2012, V. Celer, University of Veterinary and Pharmaceutical Sciences Brno.
- The role of small ORFs in the pathogenesis of porcine circovirus 2 diseases (GA524/09/0673), Czech Science Foundation, 2009-2012, V. Celer, University of Veterinary and Pharmaceutical Sciences Brno.
- Development of preparation with content of anti-sense oligonucleotides in nanoparticles for the local treatment of herpes virus infections caused by viruses HSV - 1 and HSV – 2 (FR-TI1/200), Ministry of Industry and Trade, 2009-2013, M. Krajíček, FAVEA, Ltd., R. Horváth, Genex CZ, Ltd., V. Celer, University of Veterinary and Pharmaceutical Sciences Brno.

SELECTED PUBLICATIONS

- JAROSOVA, V., CELER, V., POGRANICHNIY, R. Prevalence and age distribution of porcine torque teno sus virus (TTSuV) in the Czech Republic. *Folia microbiologica*. 2011, 56(2), p. 90-94.
- ROSENBERGOVA, K., LANY, P., POSPISIL, Z., KUBICEK, O., CELER, V. Quantification of avian influenza virus in tissues of mute swans using TaqMan real time qRT-PCR. *Veterinarni Medicina*. 2009, 54(9), p. 435–443.
- LOBOVA, D., CIZEK, A., CELER, V. The selection of single-chain Fv antibody fragments specific to Bhlp 29.7 protein of *Brachyspira hyodysenteriae*. *Folia Microbiologica*. 2008, 53(6), p. 517-521.
- MOLINKOVA, D., SKLADAL, P., CELER, V. In vitro neutralization of Equid herpesvirus 1 mediated by recombinant antibodies. *Journal of Immunological Methods*. 2008, 333(1-2), p.186-191.
- TRUNDOVA, M., CELER, V. Expression of porcine circovirus 2 ORF2 gene requires codon optimized *E. coli* cells. *Virus Genes*. 2007, 34(2), p. 199-204.

1.2. Molecular Bacteriology

Research Group Leader : Prof. MVDr. Alois Čížek, CSc.

Contact : cizeka@vfu.cz

THEMATIC RESEARCH FOCUS

Research areas

- Studies on molecular mechanisms of pathogen-host interactions
- Epidemiology of selected bacterial pathogens
- Bacterial resistance to antimicrobials and circulation of antibiotic resistance genes in animal populations and environments
- Diagnostics development

Main objectives

- To obtain novel information on the causes, mechanisms and spreading of infectious diseases in domestic animals.
- Identification of mechanisms and prevention of the circulation of zoonotic pathogens and commensal antimicrobial-resistant bacteria in the food chain.
- Definition of the role of host genetics in infectious diseases. Identification of signatures of selection in immunity-related genes based on comparative immunogenomic analysis.

CONTENT OF RESEARCH

Analysis and the prevention of the circulation of zoonotic pathogens in the food chain

Extended spectrum beta-lactamases-producing *Escherichia coli*: identification and evaluation of risk factors associated with the safety food production on farm level

Identification of the routes of spread of extended spectrum beta-lactamases-producing *E. coli* to the human population via the food chain and studying plasmid-mediated quinolone-resistance genes in commensal bacteria (*E. coli*, *Aeromonas* spp.). Recent results indicate that the risk of ESBL-producing *E. coli* spreading to the human population via food chain is relatively high. We will combine expertise in bacteriology, molecular biology and microbial epidemiology to determine the routes of spread of extended spectrum beta-lactamases-producing *E. coli* and plasmid-encoded fluoroquinolone-resistant *E. coli* to the human population via the food chain based on the identification of these *E. coli* strains in hazardous raw materials of animal origin; to define the ways of spreading of these *E. coli*, their prevalence in animal and human populations and their survival in environment including in wild animal reservoirs, and to assess risks of the spreading of these *E. coli* strains for humans, animals and environmental health. Another purpose of the project is to assess the effects of commercial probiotics and prebiotics on the induction of colonisation resistance towards resistant *E. coli* in animal intestine.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Molecular bacteriology
- Molecular bacteriology in animal pathogens

Current research infrastructure

The laboratory is presently equipped with the facilities required for the cultivation, identification and characterisation of non-fastidious bacteria.

MAIN PROJECTS

- Prevalence and subtyping study for *Escherichia coli* O157 in raw products and beef cattle (GA525/97/0373), Czech Science Foundation, 1997-1999, A. Čížek, University of Veterinary and Pharmaceutical Sciences Brno.
- *Escherichia coli* O157 and other Shiga toxin-producing serotypes: identification and analysis of risk factors of the safety food production on farm level (GA525/00/0666), Czech Science Foundation, 2000-2002, A. Čížek, University of Veterinary and Pharmaceutical Sciences Brno, P. Alexa, Veterinary Research Institute.
- Recombinant lipoprotein (BmpB) of the outer membrane of *B. hyodysenteriae* and scFv antibodies as a means of determination of asymptomatic carriers (GA524/06/1501), Czech Science Foundation, 2006-2008, A. Čížek, University of Veterinary and Pharmaceutical Sciences Brno.
- Characterisation of coliform bacteria resistant to cephalosporins and the risk assessment of antimicrobials usage for their selection (GPP502/10/P083), Czech Science Foundation, 2010-2012, M. Dolejská, University of Veterinary and Pharmaceutical Sciences Brno.
- Veterinary aspects of food safety and quality (MSM6215712402), Ministry of Education, Youth and Sports, 2005-2011, V. Večerek, University of Veterinary and Pharmaceutical Sciences Brno.

SELECTED PUBLICATIONS

- LITERAK, I., PETRO, R., DOLEJSKA, M., GRUBEROVA, E., DOBIASOVA, H., PETR, J., CIZEK, A. Antimicrobial resistance in fecal *Escherichia coli* isolates from healthy urban children of two age groups in relation to their antibiotic therapy. *Antimicrob Agents Chemother.* 2011, 55, p. 3005-3007.
- LITERAK, I., DOLEJSKA, M., RYBARIKOVA, J., CIZEK, A., STREJCKOVA, P., VYSKOCILOVA, M., FRIEDMAN, M., KLIMES, J. Highly variable patterns of antimicrobial resistance in commensal *Escherichia coli* isolates from pigs, sympatric rodents, and flies. *Microb Drug Res.* 2009, 15, p. 229-237.
- CIZEK, A., DOLEJSKA, M., NOVOTNA, R., HAAS, D., VYSKOCIL, M. Survey of Shiga toxigenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. *J Appl Microbiol.* 2008, 104(3), p. 852-860.
- LOBOVA, D., SMOLA, J., CIZEK, A. Decreased susceptibility to tiamulin and valnemulin among Czech isolates of *Brachyspira hyodysenteriae*. *Journal of Medical Microbiology.* 2004, 53, p. 287-291.
- BIELASZEWSKA, M., SCHMIDT, H., LIESEGGANG, A., PRAGER, R., RABSCH, W., TSCHAPE, H., CIZEK, A., JANDA, J., BLAHOVA, K., KARCH, H. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157: H- strains and a source of human diseases. *Journal of Clinical Microbiology.* 2000, 38(9), p. 3470-3473.



1.3. Parasitology

Research Group Leader : Prof. MVDr. Břetislav Koudela, CSc.

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THEMATIC RESEARCH FOCUS

Research areas

- Analysis of the aspects associated with diversity and host specificity of selected model groups of parasitic pathogens, their zoonotic potential and the risk of infectious diseases for human and domestic and wild animals emerging
- Development of specific and sensitive molecular tools for the diagnosis of toxoplasmosis
- Proteomic analysis of the excretory-secretory proteins of the *Trichinella* species and identification of key parasite proteins that are involved in the host-parasite interaction

Main objectives

- To obtain novel information on the causes, mechanisms and spreading of infectious diseases in domestic animals.
- Identification of mechanisms and prevention of the circulation of zoonotic pathogens and commensal antimicrobial-resistant bacteria in the food chain.

CONTENT OF RESEARCH

Analysis of the causes, mechanisms and spread of infectious diseases in domestic animals

Emerging diseases on the human-domestic animals-wildlife interface

The aim is to study principles underlying epidemiology of selected parasitic pathogens transmissible between man and animals, including improving the methods of the detection, understanding the links between molecular diversity and host specificity of selected model organisms, with emphasis on zoonotic potential.

Model situation 1: *Piroplasma* (*Babesia*, *Theileria* spp). Elucidation of host specificity and description of the barriers that prohibit the transmission of individual species / genotypes between various hosts.

Model situation 2: Protistan and helminth parasites of primates and man. The intimate evolutionary link between African great apes, such as chimpanzees and gorillas, and humans creates a unique model situation, in which we can trace the history and origin of several human infections, i.e. from the perspective of interspecific boundaries and their crossing by pathogens. This part of the project is aimed at comparing communities of protozoan and helminth parasites in population of wild gorillas and chimpanzees on the level of individuals, i.e. infra-communities, as well as host populations and to apply the molecular tools to assess the genetic diversity of studied pathogens on infra-specific level and to analyse the consequences of observed transmission patterns for emergence of diseases, but also for conservation purposes.

Model situation 3: Parasitic and viral infection in isolated populations of African domestic dogs. The study focuses on the population of domesticated animals, namely dogs, lives without prophylactic or therapeutic interventions, being exposed to direct selection pressure of complex of pathogens. The overall aim of this part of is to characterise infectious diseases in native dogs living so far without any veterinary care with Samburu/Turkana pastoralists in Turkana region N. Kenya.

Analysis and the prevention of the circulation of zoonotic pathogens in the food chain

Emerging food-borne parasites

This work package focuses mainly on nucleic acid-based and proteomic approaches for the diagnosis of the selected food-borne parasites and analysis of genetic variation among them. Among the major food-borne parasites are *T. gondii* and *Trichinella* spp. Based on two model situations, the work package aims to contribute to understanding the principles underlying the epidemiology of selected food-borne parasites transmissible between man and animals, including improving the methods of the detection.

Model situation 1: *Toxoplasma gondii*. Toxoplasmosis. Sera from food domestic and game animals will be tested for antibodies to *T. gondii*. *T. gondii* isolates from seropositive animals will be then genotyped. Genotyping data will be analysed to demonstrate the genetic diversity and geographical distribution of *T. gondii* in the Czech Republic.

Model situation 2: *Trichinella*. A global proteomics approach will be used to analyse the E/S proteins from *T. spiralis* muscle larvae. Specific *Trichinella* EST databases will be used to analyse the data.

Host genetics and comparative immunogenomics

Genetic diversity and host-pathogen interactions in specific populations of domestic dogs

Several hundred samples from Kenyan village dogs will be available for genetic diversity and association analysis of various populations and sub-populations of these dogs. For these purposes, selected infectious / parasitic diseases will be diagnosed by molecular and serological techniques: rabies, distemper and several parasite species and phenotypic classification will be made.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Parasitology

Current research infrastructure

The laboratories are well equipped to perform molecular-genetic diagnosis of parasites and advanced microscopy diagnosis.

MAIN PROJECTS

- Impact of increased contact with humans on diversity and ecology of protozoan parasites of African great apes (GA206/09/0927), Czech Science Foundation, 2009-2011, D. Modrý, University of Veterinary and Pharmaceutical Sciences Brno, M. Kváč, Biology Centre AS CR, K. Petrželková, Institute of Vertebrate Biology AS CR, I. Čepička, Charles University in Prague.
- Development of the new tools for surveillance of trichinellosis in domestic swine and wildlife animals in the Czech Republic (QH81069), Ministry of Agriculture, 2008-2012, B. Koudela, University of Veterinary and Pharmaceutical Sciences Brno, K. Kovařík, Veterinary Research Institute.
- Immunogenetic study of a house mouse hybrid zone (GA206/08/0640), Czech Science Foundation, 2008-2012, J. Piálek, Institute of Vertebrate Biology AS CR, P. Šíma, Institute of Microbiology AS CR, M. Macholán, Institute of Animal Physiology and Genetics AS CR, V. Holáň, Institute of Molecular Genetics AS CR, P. Munclinger, Charles University in Prague, D. Modrý, University of Veterinary and Pharmaceutical Sciences Brno.

SELECTED PUBLICATIONS

- FENG, Y., YANG, W., L., RYAN, U., ZHANG, L., X., KVEC, M., KOUDELA, B., MODRY, D., LI, N., FAYER, R., XIAO, L., H. Development of a Multilocus Sequence Tool for Typing *Cryptosporidium muris* and *Cryptosporidium andersoni*. *J. Clin. Microbiology*. 2011, 49(1), p. 34-41.
- SIROKY, P., KUBELOVA, M., MODRY, D., et al. Tortoise tick *Hyalomma aegyptium* as long term carrier of Q fever agent *Coxiella burnetii*-evidence from experimental infection. *Parasitol. Res.* 2010, 107(6), p. 1515-1520.
- PETRASOVA, J., MODRY, D., HUFFMAN, M., A. Gastrointestinal Parasites of Indigenous and Introduced Primate Species of Rubondo Island National Park, Tanzania. *Nt. J. Primatol.* 2010, 31(5), p. 920-936.
- JIRKU, M., VALIGUROVA, A., KOUDELA, B., KRIZEK, J., MODRY, D., SLAPETA, J. New species of *Cryptosporidium* Tyzzer, 1907 (Apicomplexa) from amphibian host: morphology, biology and phylogeny. *Folia Parasitol.* 2008, 55(2), p. 81-94.
- KORINKOVA, K., KOVARCIK, K., PAVLICKOVA, Z., SVOBODA, M., KOUDELA, B. Serological detection of *Trichinella spiralis* in swine by ELISA (enzyme-linked immunosorbent assay) using an excretory-secretory (E/S) antigen. *Parasitology Research*. 2008, 102(6), p. 1317-1320.



1.4. Food Safety

Research Group Leader : Prof. MVDr. Iva Steinhauserová, CSc.

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THEMATIC RESEARCH FOCUS

Research areas

- Cultivation and identification of foodborne pathogens
- Molecular biology characterisation of foodborne pathogens
- Monitoring of zoonotic pathogens in food chain
- Testing of resistance and survival of selected food-borne pathogens under different processing conditions

Main objectives

- To obtain novel information on the causes, mechanisms and spreading of infectious diseases in domestic animals.
- Identification of mechanisms and prevention of the circulation of zoonotic pathogens and commensal anti-microbial-resistant bacteria in the food chain.

CONTENT OF RESEARCH

Analysis and the prevention of the circulation of zoonotic pathogens in the food chain

Pathogenic and potentially pathogenic bacteria are nowadays of great interest from the food safety point of view. The contamination of food matrices will be studied by culture independent methods based on PCR and real time PCR in order to determine the impact of food processing on pathogens' presence and survival. The aims are monitoring, typing / subtyping, quantitation of selected microorganisms, and identification of genes responsible for the resistance and survival of selected food-borne pathogens under stress conditions used in the food industry.

Selected food-borne pathogens (e.g. *Campylobacter* spp., *Listeria* sp., *Salmonella* spp.) will be monitored from farm to consumers. Isolated strains from various types of farms (conventional and organic), processing plants and from patients suffering from food-borne diseases will be characterised by phenotyping and genotyping methods. The resistance and survival of selected food-borne pathogens under different processing conditions will be studied directly in food samples.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Characterisation of pathogenic isolates - determination of relations of isolated clones, assessment of resistance
- Detection of pathogens using culture and molecular techniques

Current research infrastructure

The Department of Meat Hygiene and Technology has laboratories for the isolation, growth and storage of pathogens and other infective materials. Specific laboratory units enable them to detect and characterise pathogenic microorganisms using molecular biology methods. The department has a technological laboratory with the possibility of performing technological experiments with pathogenic microorganisms and simulating real conditions.

MAIN PROJECTS

- Innovative Methods of Manufacturing, Cutting and Packaging of Meat Products, 2010, L. Steinhauser, Steinhauser, Ltd., I. Steinhauserová, University of Veterinary and Pharmaceutical Sciences Brno, Krásno Meat Industry, Inc.
- POULTRYFLORGUT – Control of the intestinal flora in poultry for ensuring the products safety for human consumers (7076), FP6-FOOD, EU, 2005-2008, I. Steinhauserová, University of Veterinary and Pharmaceutical Sciences Brno.
- Veterinary aspects of food safety and quality (MSM6215712402), Ministry of Education, Youth and Sports, 2005-2011, V. Večerek, University of Veterinary and Pharmaceutical Sciences Brno.

SELECTED PUBLICATIONS

- NEBOLA, M., BORILOVA, G., KASALOVA, J. PCR-RFLP Analysis of DNA for Differentiation of Fish Species in Seafood Samples. *Bulletin of the Veterinary Institute in Pulawy*. 2010, 54, p. 49-53.
- FAJT, Z., MODRA, H., BANOCH, T., BLAHOVA, J., SALAKOVA, A., STEINHAUSEROVA, I., SVOBODA, M. Effect of high dietary levels of Se-enriched yeast on muscle selenium content and meat quality traits – a model study in rats. *Neuroendocrinology Letters*. 2010, 31(2), p. 114-119.
- DE CESARE, A., BORILOVA, G., SVOBODOVA, I., BONDIOLI, V., MANFREDA, G. Clostridium perfringens occurrence and ribotypes in healthy broilers reared in different European countries. *Poultry Science*. 2009, 88(9), p. 1850-1857.
- BORILOVA, G., NEBOLA, M., STEINHAUSEROVA, I. Occurrence and antibiotic resistance of Campylobacter spp. isolated from pheasants (*Phasianus colchicus* spp. *torquatus*). *Archiv für Lebensmittelhygiene*. 2007, 58(5), p. 116-120.
- NEBOLA, M., STEINHAUSEROVA, I. PFGE and PCR/RFLP typing of Campylobacter jejuni strains from poultry. *British Poultry Science*. 2006, 47(4), p. 456-461.



1.5. Orthopaedics and Surgery

Research Group Leader : Prof. MVDr. Alois Nečas, Ph.D.

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THEMATIC RESEARCH FOCUS

Research areas

- Small animal orthopaedics
- Bone and joint surgery
- Arthroscopy

Main objectives

- To obtain novel information on mechanisms of infectious diseases in domestic animals.
- Definition of the role of host genetics in infectious diseases.

CONTENT OF RESEARCH

Analysis of the causes, mechanisms and spread of infectious diseases in domestic animals

The main objective is to evaluate the clinical manifestations and to make analyses of the causes of infections in surgically treated bone and joint diseases in model animals. Despite high quality care in small animal orthopaedics, we encounter, in a certain number of patients, the development of infectious disease. The aim of this study will be to analyse the causes of infections of bones and joints in surgically treated model animals and based on the obtained results, to contribute to possible reduction of infection complications in operated patients in veterinary and human medicine

Host genetics and comparative immunogenomics

The objective is to evaluate characteristics of clinical manifestations of bone and joint infections in relation to surgical procedures in animal models for purposes of genetic analysis.

KEY RESEARCH EQUIPMENT

Current research infrastructure

The laboratories are currently fully equipped for the planned surgical procedures in model animals (operating theatres, surgical equipment, instrumentation, sterilisation, anaesthesia machines etc.).

MAIN PROJECTS

- Synthesis of new biomaterials and preparation of stem cell derived cells, and their applications in for the treatment of diseases affecting human tissues derived from mesoderm: cartilage, bone, ligament and meniscus (2B06130), Ministry of Education, Youth and Sports, 2006-2011, A. Nečas, University of Veterinary and Pharmaceutical Sciences Brno, J. Motlík, Institute of Animal Physiology and Genetics AS CR, E. Syková, Institute of Experimental Medicine AS CR, P. Gál, Masaryk University, J. Jančář, Brno University of Technology.

SELECTED PUBLICATIONS

- PLANKA, L., GAL, P., KECOVA, H., KLIMA, J., HLUCILOVA, J., FILOVA, E., AMLER, E., KRUPA, P., KREN, L., SRNEC, R., URBANOVA, L., LORENZOVA, J., NECAS, A. Allogeneic and autogenous transplantations of MSCs in treatment of the physeal bone bridge in rabbits. *BMC Biotechnology*. 2008, 8(70), p. 70-78.
- MICKOVA, A., TOMANKOVA, K., KOLAROVA, H., BAJGAR, R., KOLAR, P., SUNKA, P., PLECNER, M., JAKUBOVA, R., BENES, J., KOLACNA, L., PLANKA, L., NECAS, A., AMLER, E. Ultrasonic shock-wave as a control mechanism for liposome drug delivery system for possible use in scaffold implanted to animals with iatrogenic articular cartilage defects. *Acta Veterinaria Brno*. 2007, 77(2), p. 285-289.
- JANCAR, J., SLOVIKOVA, A., AMLER, E., KRUPA, P., KECOVA, H., PLANKA, L., GAL, P., NECAS, A. Mechanical response of porous scaffolds for cartilage engineering. *Physiological Research*. 2007, 56, p. 17-25.
- KRUPA, P., KRSEK, P., JAVORNIK, M., DOSTAL, O., SRNEC, R., USVALD, D., PROKS, P., KECOVA, H., AMLER, E., JANCAR, J., GAL, P., PLANKA, L., NECAS, A. Use of 3D geometry modelling of osteochondrosis-like iatrogenic lesions as a template for press-and-fit scaffold seeded with mesenchymal stem cells. *Physiological Research*. 2007, 56, p. 107-114.
- WAGNER, K., GRIFFON, D., J., THOMAS, M., W., SCHAEFFER, D., J., SCHULZ, K., SAMII, V., F., NECAS, A. Radiographic, computed tomographic, and arthroscopic evaluation of experimental radio-ulnar incongruence in the dog. *Veterinary Surgery*. 2007, 36(7), p. 691-698.



1.6. Animal Immunogenomics

Research Group Leader : Prof. RNDr. MVDr. Petr Hořín, CSc.

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THEMATIC RESEARCH FOCUS

Research areas

- Immunogenetics and immunogenomics
- Comparative and evolutionary genomics
- Genetic resistance to infectious disease, host and pathogen interactions

Main objectives

- To obtain novel information on genetic mechanisms of infectious diseases in domestic animals.
- Definition of the role of host genetics in infectious diseases.
- Analysis of genetic diversity, evolution and selection in selected immunity-related genes based on comparative immunogenomic analysis.
- Analysis of genetic mechanisms of host and pathogen interactions.

CONTENT OF RESEARCH

Host genetics and comparative immunogenomics

The contribution of host genetic factors to infectious diseases is one of fundamental issues in understanding their pathogenesis. Comparative genomic analysis of the genes involved in resistance to model horse and dog infectious disease will be performed. The general objective of this work package is to identify candidate genes involved in host resistance to infectious disease and to analyse their diversity, evolution and selection. Two kinds of immunity-related candidate genes will be analysed: genes at the host and pathogen interface, involved in antigen presentation and recognition and immunity-related genes involved in signalling, regulatory and effector immune pathways. Their genetic diversity, evolution and selection will be analysed in selected groups of vertebrates, mostly in important groups of mammals. Among them, model populations of domestic, free-ranging and captive equids and of domestic dogs will be explored.

Analysis of genetic diversity of African village dogs may elucidate the origins of the domestic dogs. Disease association analysis and comparison with domestic dogs may reveal genes underlying mechanisms of important dog diseases. In several hundred samples from Kenyan village dogs, microsatellite, association analysis will be used for assessing genetic diversity of various populations and sub-populations of these dogs. Their relationships to other African Kenyan dogs will be studied. Specific attention will be paid to the diversity in the major histocompatibility complex genes. The polymorphism identified will be analysed for associations with resistance to infectious diseases diagnosed by molecular and serological techniques: rabies, distemper and several parasite species.

The hypothesis that resistance / susceptibility to infection following orthopaedic surgery has a genetic component and that the host defence mechanisms play an important role in the host vs. pathogen interaction will be tested. In patients with and without infection, DNA will be isolated from biological material collected and association analysis will be performed.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Host structural genomics for veterinary medicine

Current research infrastructure

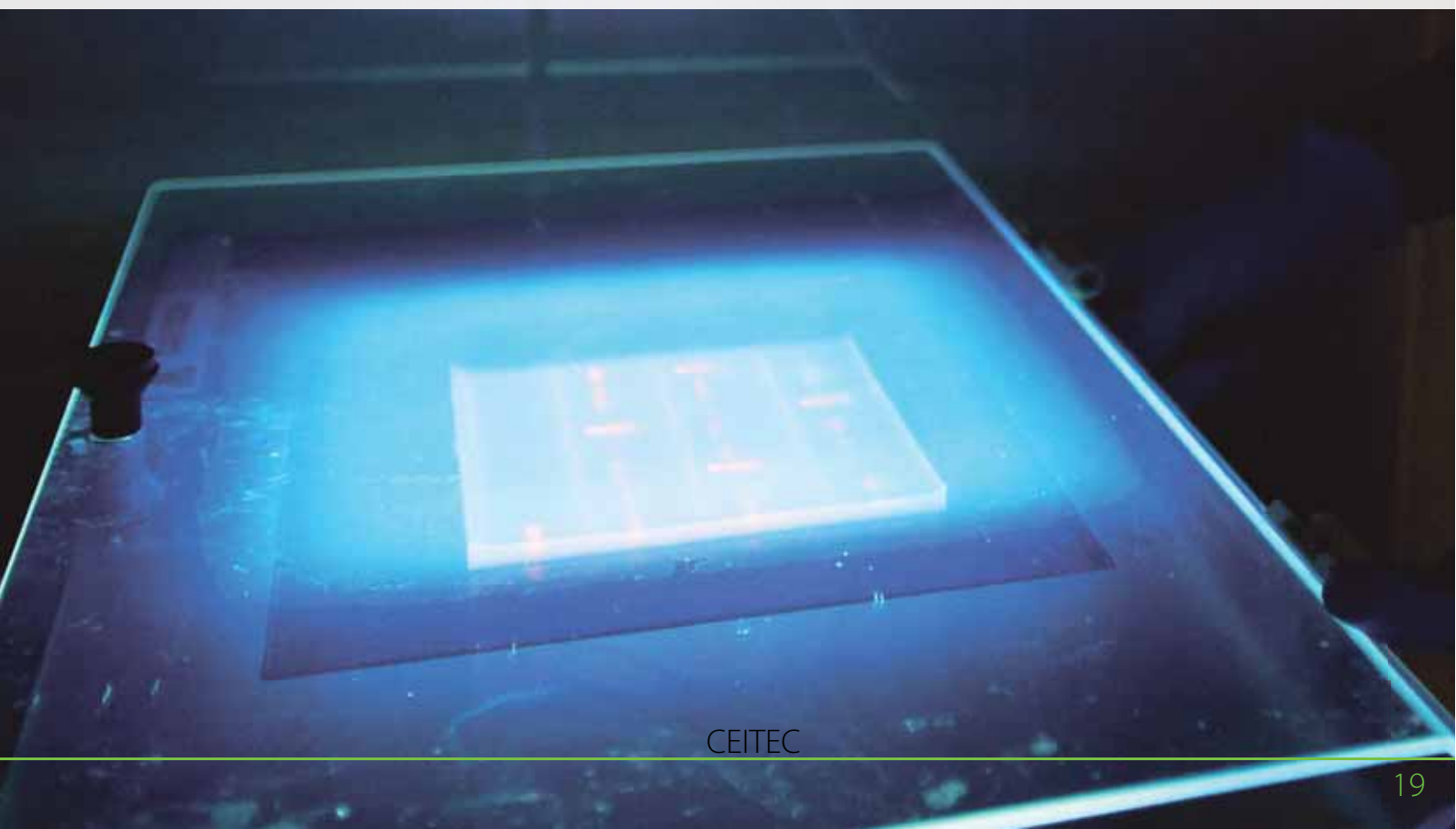
The laboratories are currently equipped to perform standard genetic DNA analyses.

MAIN PROJECTS

- Immunogenomic analysis of insect bite hypersensitivity in the Old Kladruber horse (GA524/09/1939), Czech Science Foundation, 2009-2011, P. Hořín, University of Veterinary and Pharmaceutical Sciences Brno.
- Genetic diversity and its conservation in selected horse populations in the Czech Republic (QH92277), Ministry of Agriculture, 2009-2011, P. Hořín, University of Veterinary and Pharmaceutical Sciences Brno, L. Putnová, Mendel University in Brno.
- Comparative immunogenomics of the family Equidae (GA523/09/1972), Czech Science Foundation, 2009-2012, P. Hořín, University of Veterinary and Pharmaceutical Sciences Brno, P. Musilová, Veterinary Research Institute, J. Lukeš, Biology Centre AS CR.

SELECTED PUBLICATIONS

- VRANOVA, M., ALLOGGIO, I., QABLAN, M., VYSKOCIL, M., BAUMEISTEROVA, A., SLOBODA, M., PUTNOVA, L., VRTKOVA, I., MODRY, D., HORIN, P. Genetic diversity of the class II major histocompatibility DRA locus in European, Asiatic and African domestic donkeys. *Infect Genet Evol.* 2011, 11(5), p. 1136-1141.
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- MUSILOVA, P., KUBICKOVA, S., HORIN, P., VODICKA, R., RUBES, J. Karyotypic relationships in Asiatic asses (kulan and kiang) as defined using horse chromosome arm-specific and region-specific probes. *Chromosome Res.* 2009, 17, p. 783-790.
- JANOVA, E., MATIASOVIC, J., VAHALA, J., VODICKA, R., VAN DYK, E., HORIN, P. Polymorphism and selection in the major histocompatibility complex DRA and DQA genes in the family Equidae. *Immunogenetics.* 2009, 61(7), p. 513-527.
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1.7. Animal Cytogenomics

Research Group Leader : Prof. MVDr. Jiří Rubeš, CSc.

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THEMATIC RESEARCH FOCUS

Research areas

- Comparative molecular cytogenetics and genetics of reproduction

Main objectives

- To obtain novel information on chromosomal rearrangements that have taken place during the process of evolution in the family Bovidae and Equidae by using comparative FISH.
- Determination of associations between the frequencies of chromosomally abnormal sperm, semen parameters and the reproductive outcome of the carriers of chromosomal translocations.
- Meiotic process including homologous synapsis, and frequency and distribution of recombination events will be studied on pachytene spermatocytes in animal models to elucidate the progress of meiosis in individuals with normal or abnormal karyotypes.

CONTENT OF RESEARCH

Comparative cytogenomics and the genetics of reproduction

The conservation of selected chromosome regions in different species of Equidae and Bovidae will be analysed. Changes in chromosome sizes and morphology are characteristic of the evolutionary process. Different karyotypes are usually detected even among closely related species. Many pathways have been described by which chromosomes and whole genomes change during evolution. The objectives are to obtain novel information on the chromosomal rearrangements that have taken place during the process of evolution in the Bovidae and Equidae families by using comparative FISH with painting and BAC probes to provide further insights into the evolution of the karyotypes in these families. Whole chromosome and region-specific painting probes will be prepared by laser microdissection, flow-sorting and DOP PCR. On the basis of the homologies and cross hybridisations of these sequences within the various subfamilies, phylogenetic associations between the investigated species will be specified.

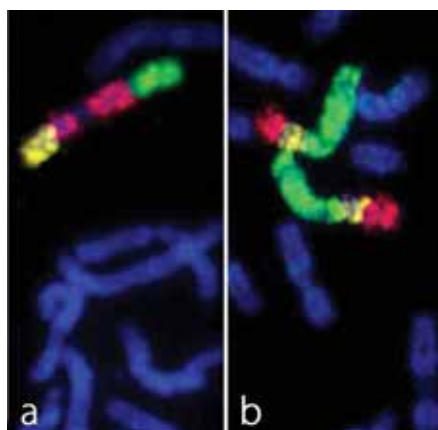


Fig. 1: ZOO-FISH hybridisation of bovine chromosomes (BTA) on the metaphase of reticulated giraffe (*Giraffa camelopardalis reticulata*): a) whole chromosome painting probes BTA 27 (yellow), BTA 20 (red), BTA 23 (green), b) BTA 28 (yellow), BTA 26 (red), BTA 1 (green).

Meiotic cell division is a complex process including recombination and equal distribution of chromosomes into gametes. The objective of this work package is to obtain information on the similarities and differences in the meiotic behaviour of chromosomes which are separate in one and fused in other related species of the Bovidae family using immunofluorescence and FISH methods. Special attention will be paid to the synapsis and recombination of sex chromosomes, especially in those species, where the fusion of one or both sex chromosomes with some autosome occurred during karyotypic evolution. The functional isolation of the autosomal and the ancestral sexual segment by intercalary heterochromatic block is necessary for the proper progression of meiosis in X-autosome translocated species. Pachytene spermatocytes from testicular samples obtained from captive-bred animals belonging to species of the Bovidae family will be analysed using immunofluorescence and FISH methods. The results obtained in species with related karyotypes will be compared. We have tests of the following species for the study: *Aepyceros melampus*, *Kobus megaceros*, *Gazella dama ruficollis*, *Oryx gazella*, *Gazella leptoceros*, *Connochaetes taurinus taurinus*, *Connochaetes gnou*, *Hippotragus niger*, *Tragelaphus imberbis* and *Taurotragus derbianus*.

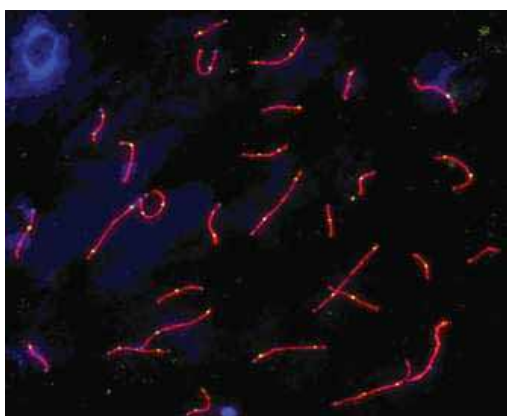


Fig. 2: Immunocytogenetics of male meiosis. Representative bovine pachytene stage spermatocyte. Synaptonemal complexes are detected by SCP3 (in red), centromeres by CREST (in blue) and sites of crossovers by MLH1 (in yellow).

The primary cause of decreased reproductive potential in carriers of translocation is incorrect meiotic segregation of chromosomal pairs included in translocation, which form a trivalent in Robertsonian translocations or quadrivalent in reciprocal translocations during the first meiotic division. Our aim is to obtain and draw a general conclusion from the information on the associations between the frequencies of chromosomally abnormal sperm and semen parameters and the reproductive outcome of the carriers of balanced translocations. The anticipated achievements will be novel information on the relationships between the frequency of chromosomally aberrant spermatozoa, the quality of spermatogenesis and the entire reproductive potential of males who are carriers of translocations. Fluorescence in situ hybridisation will be used for the investigation of meiotic segregation of normal and translocated chromosomes and the interchromosomal effect. Samples will be continuously examined by sperm analysis, FISH method and flow cytometry.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Advance microscopic study of mammalian chromosomes

Current research infrastructure

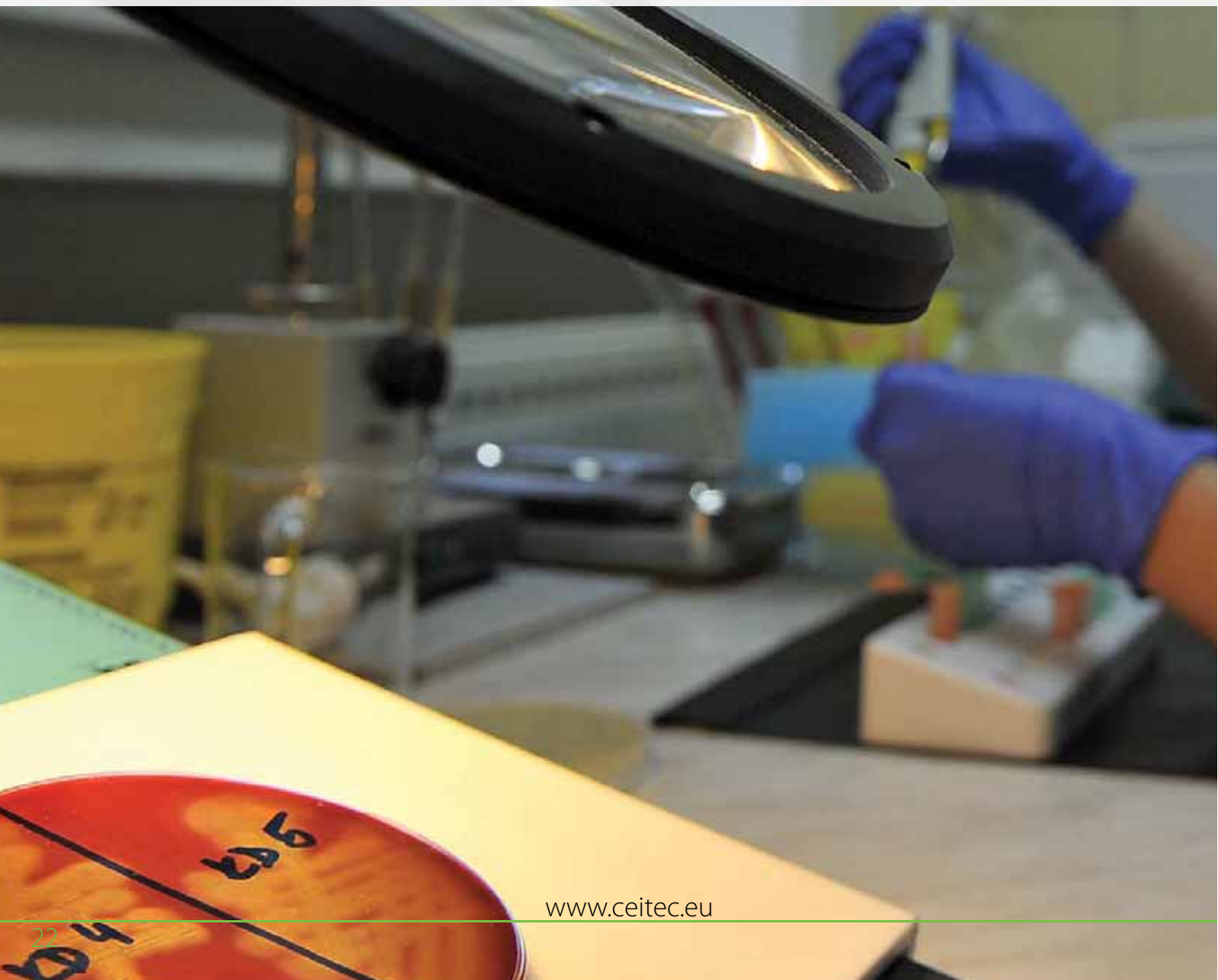
The research group has a 10-year experience with the use of microdissection and includes leading experts in this technology. Department of Genetics and Reproduction of the Veterinary Research Institute is equipped with the necessary instruments and devices for cell culture, FISH and the follow-up molecular-biological methods. The staff of the department is experienced in preparation of DNA probes for FISH on chromosomes of various species of the Bovidae and Equidae as well as in isolation of chromosome-specific centromeric repeats and germ cell analysis.

MAIN PROJECTS

- Phylogenetic relationships in the family Bovidae studied by analysis of subfamily specific DNA repeats and karyotype (GAP506/10/0421), Czech Science Foundation, 2010-2013, J. Rubeš, Veterinary Research Institute.
- Study of meiotic disturbances in men - carriers of congenital balanced translocations and their effect on the outcome of assisted reproduction (NS9842), Ministry of Health, 2009-2011, M. Vozdová, Veterinary Research Institute, R. Gaillyová, University Hospital Brno.
- Comparative study of male meiosis in members of the family Bovidae (GAP502/11/0719), Czech Science Foundation, 2011-2015, M. Vozdová, Veterinary Research Institute.

SELECTED PUBLICATIONS

- RUBES, J., RYBAR, R., PRINOSILOVA, P., VEZNIK, Z., CHVATALOVA, I., SOLANSKY, I., SRAM, R., J. Genetic polymorphisms influence the susceptibility of men to sperm DNA damage associated with exposure to air pollution. *Mutat Res.* 2010, 683, p. 9-15.
- MUSILOVA, P., KUBICKOVA, S., HORIN, P., VODICKA, R., RUBES, J. Karyotypic relationships in Asiatic asses (kulan and kiang) as defined using horse chromosome arm-specific and region-specific probes. *Chromosome Res.* 2009, 17, p. 783-790.
- RUBES, J., KUBICKOVA, S., PAGACOVA, E., CERNOHORSKA, H., DI BERARDINO, D., ANTONINOVA, M., VAHALA, J., ROBINSON, T., J. Phylogenomic study of spiral horned antelope by cross-species chromosome painting. *Chromosome Res.* 2008, 16, p. 935-947.
- VOZDOVA, M., ORACOVA, E., HORINOVA, V., RUBES, J. Sperm fluorescence in situ hybridization study of meiotic segregation and an interchromosomal effect in carriers of t(11;18). *Human Reprod.* 2008, 23, p. 581-583.
- RUBES, J., VOZDOVA, M., REZACOVA, O., ROBBINS, W., A., PERREAULT, S., D., WYROBEK, A. Stable variants of sperm aneuploidy among healthy men show associations between germinal and somatic aneuploidy. *Am J Human Genet.* 2002, 70, p. 1507-1519.



1.8. Mammalian Reproduction

Research Group Leader : [MVDr. Martin Anger, Ph.D.](#)

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THEMATIC RESEARCH FOCUS

Research areas

- Acquisition of meiotic competence in mammalian oocytes
- Regulation of chromosome segregation in mammalian meiosis
- Effect of maternal aging on chromosome segregation errors in oocytes
- Fertilisation and transition from meiosis into mitosis
- Regulation of chromosome segregation during early embryonic development

Main objectives

- To identify the essential factors important for acquisition of meiotic competence in mammalian oocytes, especially those that are conserved between species.
- To obtain detailed description of the crucial molecular mechanisms controlling chromosome segregation in mammalian oocytes.
- To determine which of those mechanisms are primarily affected by maternal aging in mammalian oocytes.
- To study the transition from meiosis into mitosis during fertilisation and accompanying changes of the cell cycle regulatory mechanisms.
- To study the regulation of chromosome segregation during early embryonic development in mammals, especially the role of checkpoint mechanisms in this process.

CONTENT OF RESEARCH

Animal models of mammalian reproduction

Mammalian oocytes are unique cells that first appear during early embryonic development. After the initial stages of the meiotic program, which also involves recombination and exchange of genetic material, oocytes are arrested at the prophase of the first meiotic division until hormonal stimulation triggers resumption of meiosis. This extremely long interruption of the meiotic program that in some species, such as humans, might last for decades can cause cell cycle disorders during later stages of the meiosis. Manifested by insufficient competence to complete meiosis or failure of chromosome segregation leading to aneuploidy, these errors accumulate with age and present serious problems for human reproduction such as increased levels of abortion or developmental disorders.

Research group will focus on two essential events in mammalian oocytes. We will study how the oocytes acquire competence to resume meiosis during growth and how the segregation of chromosomes during the resumption of meiosis is controlled. In particular we are interested in changes introduced into those events by maternal aging. Using mouse oocytes together with oocytes isolated from farm animals, we will be able to take advantage of the mouse knockout and transgenic lines and close to human meiotic progression observed in farm animals. Using techniques such as live cell confocal microscopy and kinase assays based on FRET biosensors, we will be able to study individual cells. Our results will be important for human reproduction, as well as for the improvement of techniques used in the reproduction of farm animals.

In order to obtain a complete picture about regulation of chromosome segregation at the onset of development, apart from studying this process in oocytes we will also focus our attention to the fertilisation and early embryonic development. This part of the mammalian development represents an important transition between meiotic cell cycle and mitosis and for our better understanding of the mechanisms regulating cell division and chromosome segregation and also errors, which might occur in this process, this stage of development is essential. Since the chromosome segregation during early embryonic development in human is highly error prone, our results will be valuable for preventing such errors.

Updating research infrastructure by introducing new state-of-the-art techniques to our laboratory is also one of our goals. To achieve this, we would like to introduce live imaging of oocytes and embryos in combination with kinase assays in single cell. These new techniques will not only substantially increase our chance to compete in the field for the future, but the methods and approaches developed during our study will also potentially have robust impact on reproduction medicine and animal biotechnology.

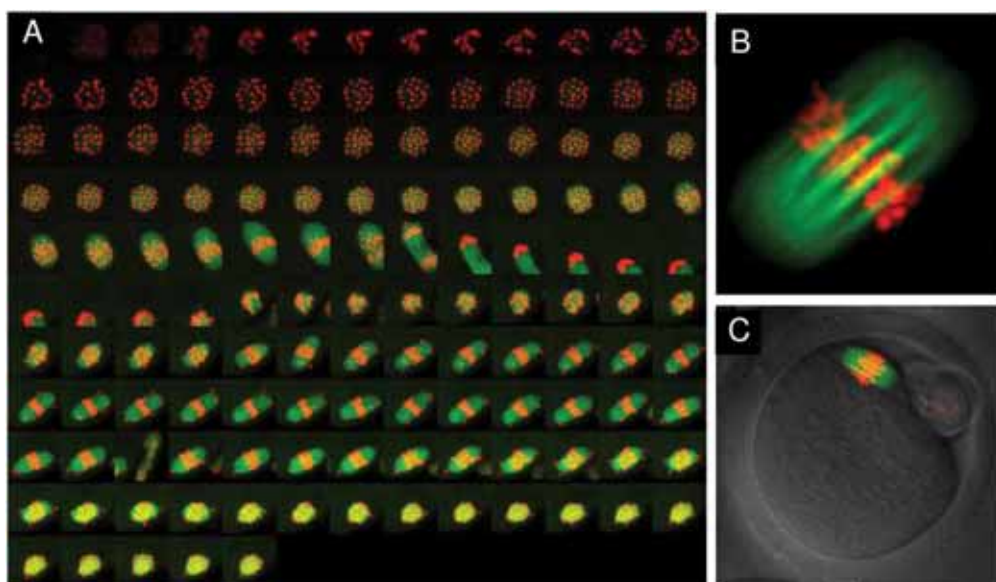


Fig.3. A) Movie frames from time-lapse experiment. Chromosomes (Red) visualised by microinjection of histone H2B fused to mCherry, spindle (Green) by microinjection of tubulin fused to EGFP, duration of the experiment 18 hours. B) High resolution image of the meiosis I spindle, chromosomes and spindle labelled as in A. C) Position of meiosis II spindle in oocyte, chromosomes and spindle labelled as in A and combined with DIC image.

KEY RESEARCH EQUIPMENT

Planned research infrastructure

Technology Units

- Analysis of mammalian gametes and embryos using live microscopy

Current research infrastructure

Our current research infrastructure includes basic equipment for in vitro culture and analysis of oocytes and embryos and basic equipment for molecular biology techniques such as RT PCR, in vitro transcription of mRNA, electrophoresis, gel documentation etc. We have also available facility for housing laboratory animals including transgenic mouse facility and facility for housing large farm animals.

MAIN PROJECTS

- Marie Curie Reintegration Grant, 2009-2011, M. Anger, Institute of Animal Physiology and Genetics, AS CR
- Regulation of chromosome segregation during meiosis (IAA501620801), Academy of Sciences of the Czech Republic, 2008-2010, M. Anger, Veterinary Research Institute, A. Hampl, Masaryk University.
- EMBO Installation Grant, 2009-2011, M. Anger, Institute of Animal Physiology and Genetics, AS CR
- The changes in important regulatory mechanisms of meiotically dividing mammalian oocytes induced by ageing (GA523/09/0743), Czech Science Foundation, 2009-2012, M. Anger, Institute of Animal Physiology and Genetics AS CR, M. Jeřeta, Veterinary Research Institute.

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- HORNAK, M., JESETA, M., MUSILOVA, P., PAVLOK, A., KUBELKA, M., MOTLIK, J., RUBES, J., ANGER, M. Frequency of aneuploidy related to age in porcine oocytes. *PLoS One*. 2011, 6(4), p. e18892.
- XU, Z., CETIN, B., ANGER, M., CHO, U., S., HELMHART, W., NASMYTH, K., XU, W. Structure and function of the PP2A-shugoshin interaction. *Molecular Cell*. 2009, 35(4), p. 426-441.
- MCGUINNESS, B., E., ANGER, M., KOUZNETSOVA, A., GIL-BERNABE, A., M., HELMHART, W., KUDO, N., R., WUENSCHKE, A., TAYLOR, S., HOOG, C., NOVAK, B., NASMYTH, K. Regulation of APC/C activity in oocytes by a Bub1-dependent spindle assembly checkpoint. *Current Biology*. 2009, 19(5), p. 369-380.
- TAM, O., H., ARAVIN, A., A., STEIN, P., GIRARD, A., MURCHISON, E., P., CHELOUFI, S., HODGES, E., ANEGER, M., SACHIDANANDAM, R., SCHULTZ, R., M., HANNON, G., J. Pseudogene-derived small interfering RNAs regulate gene expression in mouse oocytes. *Nature*. 2008, 453(7194), p. 534-538.
- KUDO, N., R., WASSMANN, K., ANGER, M., SCHUH, M., WIRTH, K., G., XU, H., HELMHART, W., KUDO, H., MCKAY, M., MARO, B., ELLENBERG, J., DE BOER, P., NASMYTH, K. Resolution of chiasmata in oocytes requires separase mediated proteolysis. *Cell*. 2006, 126(1), p. 135-146.



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