GEMINI RESEARCH TRAINING SCHOOL

Systems Biology in Maternal Communication With Gametes and Embryos

9th-13th May 2010

Opatija, Croatia

Editors:
Alireza Fazeli and Juraj Grizelj

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Web: www.cost.esf.org

WELCOME TO THE 1st GEMINI RESEARCH TRAINING SCHOOL

Message from the Chairman

GEMINI is progressing in attaining the aims and goals set in its memorandum of understanding. Now in the third year of its existence, it is a pleasure for me to welcome you to this Systems Biology Research Training School in Opatija, Croatia. Systems biology has become a fast evolving scientific discipline and a venue for close interactions between scientists involved in biology and physiology with mathematical modellers and engineers. Many biologist and engineers as well as mathematicians do not speak a common scientific language. This is, maybe, the biggest challenge that we face in systems biology; to learn to speak each others languages.

The organisation of this Research Training School is the result of collective and collaborative efforts of the GEMINI community. I hope we can further increase these collaborations and use GEMINI resources to increase our research output. Like Volos, Lansk and Alghero in Opatija plenty of time is dedicated to discussion. In the past we used this time to learn each others point of view and foster new collaborations and interactions. I hope we do the same in Opatija.

Here I would like to particularly thank Dr Juraj Grizelj for his help and contributions for the organisation of this research school. Organisation of an international research school is not an easy task and requires tremendous amount of hard work. Juraj I am thankful to you for volunteering to organise this research school and allowing us to visit your beautiful country. Here I have to acknowledge the help and support extended to us by your institution, Faculty of Veterinary Medicine, University of Zagreb, Croatia. I would like to thank all the Decanal team and particularly the Dean of the Faculty, Professor Velimir Sušić for the warm hospitality and welcoming efforts to allow us to have this research school organised in Croatia.

Mrs Sara Gottliebsen's professional and accurate service to the GEMINI community has been one of our well kept secrets of the success. Without Sara's help, we would not have been able to achieve half of what we have done so far. Sara I am thankful to you for your help and support.

I want to also take this opportunity to thank all our invited speakers/lecturers/trainers that have accepted our invitation to come to Opatija. I am very pleased to have them with us in our meeting and I look forward to hearing about the interesting science that they do. I know systems biology experts are very in demand right now. Hence, your time is very precious. Actually I learned this the hard way! Therefore, thank you very much for agreeing to teach us systems biology.

Finally, I want to thank all of you for participating in this Research Training School. I am grateful to you for your support and dedication to achieve GEMINI's goals and objectives. It is with your support and contribution to GEMINI's programs that hopefully we can make a success story of our COST ACTION.

I look forward to 3 days of intensive education. I hope after this school I will be able to get a view on how to use systems biology in my research. In addition hopefully I will leave Opatija with new friends too.

Dr Alireza Fazeli
GEMINI Chairman
May 2010

WELCOME TO OPATIJA

Dear Colleagues

It is a great pleasure to welcome you to Opatija, the point where the Mediterranean reaches most deeply into the European continent. For the reason of good geographical position and smooth climate, Opatija was in the history summer resort attended by European sovereigns, famous writers, artists and scientists. This is the reason why we decided to organize the Research Training School in “Systems Biology in Maternal Communication with Gametes and Embryo” here.

As interdisciplinary study of the component interactions and properties of biological systems, we are honoured to attend all the researchers arriving from many different countries, presenting their work as lecturers or presenters of oral communications and posters, at the Systems Biology Training School.

I would like to express a special thank you to the Decanal team of the Faculty for Veterinary Medicine Zagreb, celebrating its 90th Anniversary in 2009, for their support.

We hope you have a pleasant stay and meet people sharing the same research passion.

Best wishes

Juraj Grizelj
Local Research Training School Organiser

GEMINI RESEARCH TRAINING SCHOOL

SYSTEMS BIOLOGY IN MATERNAL COMMUNICATION WITH GAMETES AND EMBRYO

OPATIJA, CROATIA

10th – 12th May 2010

Programme

Sunday 9th May 2010

13:00 Registration & Informal get together & Reception

18:00 Close of Registration

20:00 Dinner

Monday 10th May 2010

09:00-09:15 Opening and Welcome from the Local Organiser
Juraj Grizelj, Local Organiser

09:15-09:30 Welcome and Overview of the School's Programme
Alireza Fazeli, GEMINI Chairman

09:30-10-10 From *in vivo* to in silico models in maternal communication with gametes and embryo
Alireza Fazeli, Department of Human Metabolism, University of Sheffield, UK

10-10-10:50 An overview of high-throughput genomic analysis platforms
Carmen Almiñana, Department of Human Metabolism, University of Sheffield, UK

10:50-11:20 Coffee Break (plus poster viewing)

11:20-12:00 An overview of high-throughput proteomic analysis platforms I
Phillip Wright, ChELSI Institute, Department of Chemical & Process Engineering, University of Sheffield, UK

12:00-12:40 What is systems biology (from a biologist's point of view)
François Iris, Bio-Modelling Systems

12:40-14:10 Lunch and Poster Session

14:10-14:50 Project Case Study:
Does maternal diet during preimplantation development regulate maternal-embryonic communication of nutrient availability through placental development and function? A Systems Biology Approach
Emma Lucas, University of Southampton, UK

14:50-15:30 Project Case Study:
A Systemic approach to characterise and define an in vitro model to study the female reproductive tract interaction with spermatozoa
Ahmed Aldarmahi, University of Sheffield, UK

15:30-16:10 Making inroads into the Drosophila female reproductive system
Yael Heifetz, The Hebrew University, Israel

16:10-16:40 Tea Break (plus poster viewing)

16:40-17:40 Computational modelling of natural systems I
Richard Clayton, Department of Computer Science, University of Sheffield, UK

17:40-18:40 Poster Walk

18:40-19:00 Closing discussion

20:00 Dinner

Tuesday 11th May 2010

09:00-09:40 Part I: How to develop a strategy for a systemic approach in studying maternal communication with gametes and embryo?
Jean-Pierre Ozil, INRA, France

09:40-10:20 Heuristic models: what they do and what can be expected?
François Iris, Bio-Modelling Systems

10:20-10:40 Student Presentation:
Endometrial and myometrial expression of aromatase P450 and the effect of IL1β and IL6 on estradiol 17β secretion in porcine uterine tissues during early pregnancy
Anita Franczak, University of Warmia and Mazury, Poland

10:40–11:10 Coffee break (plus poster viewing)

11:10-11:50 An overview of high-throughput proteomic analysis platforms II
Phillip Wright, ChELSI Institute, Department of Chemical & Process Engineering, University of Sheffield, UK

11:50-12:30 Computational programming and languages in simple words for biologists
Mark Burkitt, University of Sheffield, UK

12:30–14:00 Lunch (plus poster viewing)

14:00-14:40 Project Case Study:
Embryo – endometrium interactions at the time of implantation
Signe Altmäe, University of Tartu, Estonia

14:40-15:20 Project Case Study:
A systems biology approach for understanding maternal communication with embryo
Carmen Almiñana, University of Sheffield, UK

15:00-15:20 Project Case Study:
Expression of key components of RISC required for biogenesis and performance of miRNA in the porcine endometrium during early embryo-maternal cross-talk
Kamil Krawczynski, Institute of Animal Reproduction and Food Research Polish Academy of Science, Poland

16:00–16:30 Tea break (plus poster viewing)

16:30-17:30 Computational modelling of natural systems II
Richard Clayton, University of Sheffield, UK

17:30-17:50 Student Presentation:
Effect of prostaglandin E2 synthase and prostaglandin F2α synthase silencing on the expression of prostaglandin E2 receptor in endometrial cells

Agnieszka Waclawik, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland

17:50-18:10 Student Presentation:
Expression and localization of the chaperone protein MRJ in pig gametes and gonads
Georgia Pennarossa, University of Milan, Italy

20:30 ***Dinner***

Wednesday 12th May 2010

09:00-09:40 Systems biology from subcellular level to organ: Using heart as an example
Richard Clayton, Department of Computer Science, University of Sheffield, UK

09:40-10:20 An overview of bioinformatic analysis and database tools used in systems biology
François Iris, Bio-Modelling Systems

10:20-11:00 What is systems biology (from an Engineer's point of view)?
Phillip Wright, ChELSI Institute, Department of Chemical & Process Engineering, University of Sheffield, UK

11:00–11:30 Coffee break (plus poster viewing)

11:30-12:10 Part II Cellular dynamics using microscopy and microfluidics for driving system biology
Jean-Pierre Ozil, INRA, France

12:10-12:30 Student Presentation:
Regulation of non-classical Major Histocompatability Complex Class I mRNA expression in bovine embryos
Abdullah Al Naib, University College Dublin, Ireland

12:30–14:00 Lunch (plus poster viewing)

14:00-14:40 Project Case Study:
miRNAs play a role in the regulation of the Drosophila female mating response
Ido Carmel, The Hebrew University, Israel

14:40-15:20 Project Case Study:
Identification and functional analysis of specific proteins synthesized in the bovine uterus during the early development
Eva Correia, Centro de Biotecnología Animal – SERIDA ; Área Genética y Reproducción Animal, Spain

15:20-16:00 Project Case Study:
Using Computational Systems Biology to Investigate Sperm Navigation and Transport in the Female Reproductive Tract
Mark Burkitt, University of Sheffield, UK

16:00–16:30 Tea break (plus poster viewing)

16:30-17:30 Computational modelling of natural systems III
Richard Clayton, Department of Computer Science, University of Sheffield, UK

17:30-18:20 Closing Discussion

20:30 Dinner

Thursday 13th May 2010

09:00 Day Excursion

- The excursion will be decided later and will be approximately Euro 50 paid directly by participants.

INVITED

SPEAKER

ABSTRACTS

Monday 10th May 2010
09:30-10:10

Dr Alireza Fazeli
Department of Human Metabolism, University of Sheffield, UK

From *in vivo* to in silico models in maternal Communication with gametes and embryo

It is important to understand the complex interactions between gametes or embryos and the maternal genital tract. It is clear that we require different experimental models for understanding different process involved in maternal communication with gametes and embryo. The selection of the right model is an important task to undertake, and despite many new developments in this area, an ideal model system has not yet been developed.

In this presentation, I will focus on how the most appropriate model species and model system can be selected. Initially an overview of some of the experimental models used will be presented, each with its particular advantages and disadvantages. Thereafter I will discuss some selection criteria that need to be considered when a model is selected. These selection criteria need to be based on the evaluation of the aim of the experiment, the tools that are available to the scientist, and the ethics that are involved in working with particular animal species and model systems.

Society and politics direct scientists to “Refine, Reduce, and Replace” the use of experimental animals, which means that the use of in vivo models is increasingly being discouraged. An in vivo model allows experimentation in the full biological environment of a living organism. In contrast with in vivo models, in vitro models are less complex and are abstracts of in vivo systems, leading often to results that are different from the in vivo situation.

If all the components of a complex biological system were understood and were re-created as individual smaller models in a computer, it is may be possible to create in silico models that would completely represent the complexity of in vivo models. In silico models seems to be the natural departure from in vivo, in situ, and in vitro model approaches. In this presentation I will discuss numerous advantages that this approach can bring to studying maternal interaction with gametes and embryo. In silico models and simulations are perhaps the only true alternative method to animal experimentation.

Monday 10th May 2010
10:10-10:50

Dr Carmen Almiñana
Department of Human Metabolism, University of Sheffield, UK

An overview of high-throughput genomic analysis platforms

Medical and animal sciences are increasingly applying high-throughput genomic technologies to study gene expression, molecular interactions and the cellular environment. These technologies have changed our ability to study the molecular basis of cells and tissues in health and diseases, giving us a new comprehensive and different view of biology.

In this lecture to better understand what genomics is, the so-called structural genomics and functional genomics, will be defined, followed by a walk through the most important current technologies, from sequencing to the study of gene expression and function, to end with the next generation of high-throughput techniques.

Emerging technologies have provided with methods to quickly analyse genes and have enabled the acquisition of large genomics data sets. The first high-throughput techniques to be developed were sequencing methods. Since then, a great number of genomes from different species have been sequenced. The availability of a genome sequence leads to questions on gene function and serve as the basis for a wide variety of functional genomics approach, mainly concerned with patterns of gene expression during various conditions.

Microarrays have become one of the most popular tools in the hands of biologists in the past few years. Microarrays are ideally designed for analyzing thousand of genes in a great number of samples allowing large scale genomics studies. A variety of microarrays-based platforms and techniques have been developed in the recent years, allowing the application of this high-throughput technology from human to a large number of species, where enough information on sequences and annotation exist. As an alternative to the microarrays, different group of high-throughput techniques has also been developed. One of such techniques is the well-known Serial Analysis of Gene Expression (SAGE) method. This technology does not require of the existence of known DNA/RNA sequence. In the same line, Massively Parallel Signature Sequencing (MPSS) has appeared most recently. A new generation of high-throughput sequencing technologies has emerged allowing to study genome sequence variation, DNA methylation, protein-DNA interactions, transcriptome sequencing, alternative-splicing, small RNAs and mRNA regulation. These ultra high-throughput sequencing techniques will open a wide range of possibilities in the life science.

In this lecture, I will discuss those and other high-throughput technologies, giving an overview of high-throughput genomic analysis platforms. In addition, I will discuss some of the advantages and limitations of the current techniques as well as the initial parameters to consider when designing an experiment.

Monday 10th May 2010
11:20-12:00

Professor Phillip Wright
ChELSI Institute, University of Sheffield, UK

An overview of high-throughput proteomic analysis platforms I

Nowadays it is generally considered that we have moved away in many regards from conventional 2-dimensional gel electrophoresis for large-scale proteome profiling/analysis. High-throughput workflows for proteomic analysis typically involve mainly orthogonal liquid-based separations, and occasionally 1-dimensional gel separation followed by liquid chromatographic separation. The resulting separation products (usually peptides) are then subjected to tandem mass spectrometry for identification. This lecture will focus on the different workflows available for high-throughput proteomics and the advantages and disadvantages of each. In particular, it will demonstrate some specific issues related to different biological situations, and what may be used to maximize the identifications of peptides and proteins.

Monday 10th May 2010
12:00-12:40

François Iris
Bio-Modelling Systems

What is systems biology (from a biologist's point of view)

By focusing on how functions arise from dynamic interactions, systems biology addresses the missing links between molecules and physiology. Top-down systems biology identifies molecular interaction networks on the basis of correlated molecular behaviour observed in genome-wide "omics" studies. Bottom-up systems biology examines the mechanisms through which functional properties arise in the interactions of known components (Bruggeman and Westerhoff, 2007).
Hence, the aim of systems biology is to describe how living systems function and how they can make adaptive choices. But having said that, what can we do?

The survival of multicellular organisms depends upon the ability of cells and tissues to communicate among themselves as well as with their environment. Thus, cellular systems have evolved signalling pathways in which extracelluar cues trigger a cascade of intracellular information flow, causing physical, chemical and geographical modifications of intracellular components, gain as well as loss of functional capabilities. These affect a plethora of mechanisms culminating in phenotypic responses that are largely modulated by the intracellular state at the time signalling events were initiated. But such pathways cannot be conceptualised as discrete entities responding to specific triggers. There is considerable inter-pathways cross talk leading to network complexities which, to be correctly apprehended, require global, multivariate approaches.

Bayesian networks and models, a form of graphical representation of biological mechanisms, can represent probabilistic dependence relationships amongst multiple interacting components and thus illustrate the effects of pathways components upon each other in the form of influence diagrams. Thus, Bayesian networks can represent complex stochastic/non-linear relationships amongst multiple interacting components while accommodating the noise/fuzziness inherent to biological data. However, because of the statistical nature of the outputs, Bayesian approaches imperatively require multiple observation of a same phenomenon as represented by quantitative data. This results in very sever limitations, particularly when applied to multicellular processes, the physiological understanding of which require that the mechanisms be reverse-engineered from the available data.
Multiple direct and indirect interactions between very different cellular systems and between multiple organs must therefore be simultaneously considered. This is further exacerbated by physiological heterogeneity. As a consequence of individual-specific genetic (SNPs) and epigenetic effects, functionally different mechanisms can present similar phenotypic appearances while mechanisms with well identified biological roots often present considerable variability in phenotypic outcome amongst individuals, including between siblings. Cumulatively, all these factors play havoc upon analytical approaches that largely rely upon statistical coherence within and between datasets. This leads to a situation where so many different models could fit the same data that both iterative frameworks and probability distributions become ineffective. One is then faced with the clear impossibility of having to enumerate and test all possible models associated with a set of hypotheses.

But another approach is possible. While mathematical (Bayesian) modelling starts from quantitative data to produce models capable of reiterating this data and predict the outcome of a different experimental paradigm, heuristic modelling starts from accumulated knowledge to produce a model capable of describing the mechanisms that generated the observed experimental data and predict their modifications associated with a different outcome.

This lecture will develop these aspects and explain the approaches and the methods whereby one can harness heuristic frameworks to produce mutlti-levels (from intracellular mechanisms to whole organ/tissues physiology) models of complex biological processes that can then be independently tested and biologically validated or refuted.

Monday 10th May 2010
16:20-17:20

Dr Richard Clayton
Department of Computer Science, University of Sheffield, UK

Computational modelling of natural systems I

In this session we will introduce the concept of modelling, how models underpin virtually every activity in physical sciences and engineering (for example design and build of the Airbus A380 airliner), and how these ideas can be applied to biology. We will cover the following:

- Computational modelling using agents and continuum models;
- Computational codes and resources;
- Simple models of population dynamics;
- Steady state solutions;
- Oscillations;
- Phase space;
- Models of (bio)chemical reactions.

Tuesday 11th May 2010
09:00-09:40

Professor Jean-Pierre Ozil
INRA, France

Part I: How to develop a strategy for a systemic approach in studying maternal communication with gametes and embryo?

One of the objectives of COST action FA 0702 is to increase our knowledge base regarding the application of "Systems Biology" approaches in the understanding of maternal interactions/communications with gametes and embryos with the aim of acting on the system on purpose.

A stringent particularity of maternal interaction with gametes and embryos is the fact that many consequences of the interactions of gametes with their environment (in vitro or in vivo) during the periods of fertilization and early pre-implantation stages are not immediate. Modifications of embryo functioning during the early stages are usually observable only after a long period of time, for example after birth and sometimes not until the adult age. Data from animal studies have clearly shown that IVF and some embryo culture media are associated with changes in the expression of imprinted genes and can result in "**large offspring syndrome.**" It has also been demonstrated that the phenotypes of key somatic cell types affecting fetal and postnatal hepatic, cardiovascular and neuronal functions are most susceptible to these early developmental influences. This long delay between a proximal impact (environment) and a distal response of the system (animal phenotype) constitutes a significant difficulty that complicates systems biology approaches because the developmental responses over time are unlikely to be linear, reflecting the permanent input of the series of autocatalytic cycles, feedback circuits and other manifestations of circular causalities and adaptive pathways involved.

In fact scientific investigations of what causes these long term effects in the context of developmental biology face a quandary: if the developmental processes are interrupted at time "**t"** to get a picture of the molecular status of the cell, it is impossible to foresee the future of that cell at time "**t+n"** because the developmental processes have been stopped or altered. Conversely, if the developmental processes are allowed to proceed uninhibited, it is impossible to obtain clues about the molecular source phenomena leading to the phenotype. In addition, the minute amounts and numbers of interacting cellular components make direct analysis of the impact of the cellular environment on cell metabolism almost impossible.

Many scientists have investigated the early events during fertilization that can affect the long-term development and health of the offspring. But how do the initial states, leading to different outcomes, arise in the process of development? How does an experimental selection of specific initial conditions lead to precise rules for long-term differentiation? Can we approach these questions by conjugating system manipulation which requires high precision, with modelling approaches?

In this session, we examine new methods and tools showing that interaction within a biochemical pathway provide the leverage for a systemic approach**.** Driving such pathways makes it possible to orientate cell functioning regimes without perturbations, thereby providing means by which we can take direct action upon physiological regulations and correlate the variations in cell functioning at time "**t**" with developmental plasticity at time "**t+n."**

We describe a concrete example by using global functional properties of the mouse zygote that make it possible to draw phenomenological correlations between proximate and distal events. We demonstrate that it is possible to both **act on egg development** from outside the cell and **observe** the result of our actions in real time over the long term trajectory.
We will discuss whether a **systemic approach** that encompasses **functional** aspects can be implemented to investigate the complex biochemical regulation processes triggered by fertilization, the functioning of which determine the egg's destiny.

Tuesday 11th May 2010
09:40-10:20

François Iris
Bio-Modelling Systems

Heuristic models: What they do; What can be expected

Heuristics is a problems solving approach evaluating each step in a process, searching for satisfactory solutions rather than for optimal solutions, using all available qualitative information instead of quantitative information.

The role of holistic modelling is to qualitatively define the nature and sequence of functional events and mechanistic modifications that associate with the development of physiological/pathological phenomena with the aim of predicting the most probable behaviours, the most likely outcome and the best-fit intervention scenarios. Hence, the aim of heuristic models is to provide biologically valid solutions to specified problems**.** To these effects, qualitative inference of interaction networks and physiological responses are constructed using analytical procedures (CADI™) that associate algorithmics and heuristics. The logic behind this model-building approach does not assume functional linearity and the components of a model do not incorporate solely what is known. Since the modeling process implements strict negative selection of hypotheses, models arising from this procedure contain elements that have never been described but cannot be refuted by current knowledge and/or available biological data. As a result, these heuristic models predict responses and behaviours hitherto undetected.

The outputs take the form of mutlti-levels biological pathways and mechanisms interactions maps (predictive biological model) associated with in-depth textual documentation disclosing the scientific basis behind the model, together with the most relevant sources of information utilised (detailed references list).

This lecture will present in details four different types of heuristic models addressing 1) an embryoglogical process closely associated with sex differentiation, 2) the mechanism of action of a candidate treatment for breast cancer, 3) a novel approach to fighting multi-resistant bacterial infections without recourse to antibiotics or vaccines, and 4) the modes of pathogenesis and disease progression of a fatal neurodegenerative disorder.

Individually, these models directly led to 1) the explanation and publication of the mechanisms driving a complex developmental process that had remained obscure for over 60 years, 2) the curtailment of a drug development program that would have resulted in a costly failure, 3) the development and patenting of 3 novel technologies and the creation of a BioPharma Company specialised in biodefense/biosecurity, and 4) the first ever explanation of the mechanisms driving a neurogegenerative disease (rewarded by a US industrial award), the discovery and patenting of a novel form of treatment applicable to multiple cognitive diseases and the creation of a BioPharma Company specialised in psychiatric therapeutics.

Thus, what **can** reasonably be expected from heuristic models are biologically « robust » and « objective »mechanisms & biomarkers (clinical & therapeutic) defined by physiological relevance and not by statistical occurrence. What **cannot** be expected however are 1) Models that exhaustively describe all aspects of a physiological process; 2) Mechanisms & biomarkers universally valid in a pathology defined on the basis of clinical symptoms (semiology); 3) Mechanisms & biomarkers that will systematically be monitored from body fluids (blood and/or plasma and/or serum and/or urine) and, 4) Mechanisms & biomarkers with ideal characteristics (immediate detection/release at pathology onset + activity/release proportional to disease extent + long half-life +absent from unaffected tissues [zero baseline] + rapid, simple, accurate and inexpensive detection in all relevant individuals + etc…).

Tuesday 11th May 2010
10:50-11:30

Professor Phillip Wright
ChELSI Institute, University of Sheffield, UK

An overview of high-throughput proteomic analysis platforms II

For high-throughput proteomics, the key initial developments focused on developing tools to separate 1000s of proteins and 10s-100s of thousands of peptides. This process is still far from complete, but we have made great achievements in this area, and 1000s of protein identifications are now becoming routine. Now that these achievements have been made, the proteomics community in recent years has been increasingly focused on quantitation of proteins using these workflows. Tools using various isotope labeling strategies are key to high-throughput quantitation. This presentation will focus on techniques using metabolic labeling (eg SILAC) and isobaric mass tagging (iTRAQ and TMT) for quantitation. The advantages and limitation of each workflow will be discussed. Finally some comments on data handling and analysis will be presented.

Tuesday 11th May 2010
11:30-12:10

Mr Mark Burkitt
Department of Computer Science, University of Sheffield, UK

Computational Programming and Languages in Simple Words

Modern techniques for measuring biological systems typically generate large quantities of data. Analysing these data by hand is no longer possible, and modern spread sheet applications are not able to easily cope with larger data sets.

Analysis of data is not the only use for modern computers, which can be used to create computational simulations of biological systems, which allow virtual experiments to be conducted to investigate aspects of a system which may not be possible using in vivo or in vitro techniques due to legal, financial or ethical constraints.

Specialist commercial software is available to help with this, however often this does not do exactly what is required, or is prohibitively expensive. Sometimes the only option is to write a computer program from scratch. A large number of programming languages exist, each with it's own advantages and limitations. As with spoken languages, programming languages have their own vocabulary, syntax structure and conventions which can only be mastered with practice.

A basic overview of how computer programs work and how they are written will be provided. Using simple examples, the basics behind modern programming languages, and how they are used to create programs that can be run on a computer will be explored. This will cover the basic data types, loops and code constructs, functions and classes. A comparison of the different types of programming languages will be provided, along with a description of the different factors which should be considered when deciding on which language to use. The tools available to help with programming will also be described. Finally, a brief description of how large and complex programs can be written will be provided.

Tuesday 11th May 2010
15:50-16:50

Dr Richard Clayton
Department of Computer Science, University of Sheffield, UK

Computational modelling of natural systems II

In this session we will introduce the way that spatial information can be included in a model, and extend our analysis of reaction kinetics. We will look at:

- How Brownian motion of atoms and molecules can be effectively modelled using a diffusion equation;
- Extending mathematical models of diffusion by adding in terms to describe reaction and advection;
- Modelling a signalling pathway;
- Assumptions that underlie models of systems;
- Parameters and parameter sensitivity;
- How do we know that a model is correct?

Wednesday 12th May 2010
09:00-09:40

Dr Richard Clayton
Department of Computer Science, University of Sheffield, UK

Systems biology from subcellular level to organ: Using the heart as an example

The heart is an electromechanical pump, where a propagating wave of electrical activity – the action potential – acts to initiate and co-ordinate contraction. Each heartbeat therefore involves processes at spatial scales ranging from changes in the conformation of ion channels at the molecular scale, to force generation at the whole organ scale. Failure of any part of this system can result in cardiac arrhythmias, which can result in sudden cardiac death. In this talk, I will describe why modelling is becoming a valuable tool for investigating cardiac arrhythmias, and explain how a computational model of electrical activity in the heart can be constructed, and extended to include coupling of electrical and mechanical function.

Wednesday 12th May 2010
09:00-09:40

François Iris
Bio-Modelling Systems

An overview of bioinformatic analysis and database tools used in systems biology

High-throughput studies of biological systems are accumulating considerable masses of omics information. The challenge is to create clear, meaningful and integrated understanding and insight without being overwhelmed by the complexity of the data. A plethora of tools and database resources aiming to foster a variety of analytical approaches have been developed. There are currently over 300 web resources (see http://pathguide.org/) providing access to many thousands of pathways and networks that document millions of interactions between proteins, genes and small molecules. Although largely Bayesian, these tools are very diverse and can be broadly divided into two partly overlapping categories; The first consisting of tools focused on automated methods for interpreting and exploring large biological networks, and the second consisting of tools focused on assembly and curation of pathways. Many of these tools are tightly integrated with public databases, thus allowing users to visualize and interpret their own data in the context of previous knowledge. However, while efficiently addressing unicellular systems, these tools and databases are extremely limited in their abilities to allow multicellular systems to be coherently addressed. In most cases, data representation is approached from an "unsupervised" standpoint, making no, or few prior assumptions about the contextual validity of the links between components that might correlate. When approached globally, using these widely applied "unsupervised" analytical methods, the size and complexities of biological networks increase dramatically, leading to scarcely utilisable analytical outputs. This lecture will provide an overview of the tools, analytical platforms and databases most frequently utilised in studies addressing biological mechanisms at the systemic level, together with an assessment of the results generated.

Wednesday 12th May 2010
10:20-11:00

Professor Phillip Wright
ChELSI Institute, University of Sheffield, UK

What is systems biology (from an Engineer's point of view)?

Systems biology is: "…the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life. Instead of analyzing individual components or aspects of the organism, such as sugar metabolism or a cell nucleus, systems biologists focus on all the components and the interactions among them, all as part of one system." (The Institute for Systems Biology, www.systemsbiology.org) As part of this, it is vital that there is an iterative relationship between model and experimentation, ie the "wet lab" and the "dry lab". If it does not contain both these things working together, then it is NOT systems biology. Engineers often have very applications orientated views on systems biology. This is even more so with the advent of the emerging new field of synthetic biology. This presentation will address both these fields and show how they can be useful for generation of new products and assessing, understanding and talking problems in human health.

Wednesday 12th May 2010
11:30-12:30

Professor Jean-Pierre Ozil
INRA France

Part II Cellular dynamics using microscopy and microfluidics for driving system biology

One of the objectives of COST action FA 0702 is to increase our knowledge base regarding the application of "Systems Biology" approaches in the understanding of maternal interactions/communications with gametes and embryos with the aim of acting on the system on purpose.

A stringent particularity of maternal interaction with gametes and embryos is the fact that many consequences of the interactions of gametes with their environment (in vitro or in vivo) during the periods of fertilization and early pre-implantation stages are not immediate. Modifications of embryo functioning during the early stages are usually observable only after a long period of time, for example after birth and sometimes not until the adult age. Data from animal studies have clearly shown that IVF and some embryo culture media are associated with changes in the expression of imprinted genes and can result in "**large offspring syndrome.**" It has also been demonstrated that the phenotypes of key somatic cell types affecting fetal and postnatal hepatic, cardiovascular and neuronal functions are most susceptible to these early developmental influences. This long delay between a proximal impact (environment) and a distal response of the system (animal phenotype) constitutes a significant difficulty that complicates systems biology approaches because the developmental responses over time are unlikely to be linear, reflecting the permanent input of the series of autocatalytic cycles, feedback circuits and other manifestations of circular causalities and adaptive pathways involved.

In fact scientific investigations of what causes these long term effects in the context of developmental biology face a quandary: if the developmental processes are interrupted at time "**t"** to get a picture of the molecular status of the cell, it is impossible to foresee the future of that cell at time "**t+n"** because the developmental processes have been stopped or altered. Conversely, if the developmental processes are allowed to proceed uninhibited, it is impossible to obtain clues about the molecular source phenomena leading to the phenotype. In addition, the minute amounts and numbers of interacting cellular components make direct analysis of the impact of the cellular environment on cell metabolism almost impossible.

Many scientists have investigated the early events during fertilization that can affect the long-term development and health of the offspring. But how do the initial states, leading to different outcomes, arise in the process of development? How does an experimental selection of specific initial conditions lead to precise rules for long-term differentiation? Can we approach these questions by conjugating system manipulation which requires high precision, with modelling approaches?

In this session, we examine new methods and tools showing that interaction within a biochemical pathway provide the leverage for a systemic approach**.** Driving such pathways makes it possible to orientate cell functioning regimes without perturbations, thereby providing means by which we can take direct action upon physiological regulations and correlate the variations in cell functioning at time "**t**" with developmental plasticity at time "**t+n."**

We describe a concrete example by using global functional properties of the mouse zygote that make it possible to draw phenomenological correlations between proximate and distal events. We demonstrate that it is possible to both **act on egg development** from outside the cell and **observe** the result of our actions in real time over the long term trajectory.
We will discuss whether a **systemic approach** that encompasses **functional** aspects can be implemented to investigate the complex biochemical regulation processes triggered by fertilization, the functioning of which determine the egg's destiny.

Wednesday 12th May 2010
16:30-17:30

Dr Richard Clayton
Department of Computer Science, University of Sheffield, UK

Computational modelling of natural systems III

In this final session we will examine the Hodgkin Huxley model of the squid giant axon, which was one of the earliest models of a biological system, and has proved to be extremely influential. We will cover:

- Experimental work on the squid giant axon;
- How the Na^+ channel and K^+ channel kinetics were described and parameterised based on experimental data;
- Properties of the Hodgkin Huxley model;
- Insights into the squid giant axon from the model;
- Legacy of the Hodgkin Huxley model;

Finally, we will wrap up by reviewing other areas of computational systems biology, the future of computational systems biology, and some the difficult challenges in computational systems biology.

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16	Bartosz Wojciechowicz PhD Student Poland	Presence of P450 aromatase (P450arom) and production of 17β-estradiol (E2) by porcine endometrium and myometrium during early pregnancy and the estrous cycle (days 10 to 11, 12 to 13 and 14 to 16).
17	Agata Zmijewska PhD Student Poland	The influence of interleukin-1β on synthesis and secretion of prostaglandins by corpora lutea in cyclic and pregnant pigs

POSTER

PRESENTATIONS

Poster 1

Abdullah Al Naib
S. Mamo, P. Lonergan, T. Fair
PhD Student
University College Dublin, Ireland

Regulation of non-classical Major Histocompatability Complex Class I mRNA expression in bovine embryos

Regulation of expression of the class-I major histocompatability complex at the maternal foetal interface may play a critical role in embryo survival and the establishment of pregnancy in cattle. However, information concerning immunoregulation of implantation in cattle remains quite limited. Therefore, our current research is concerned with characterizing the expression and regulatory effect of a number of immune factors in the developing bovine embryo. To this end, cumulus oocyte complexes (COCs) were recovered from ovarian follicles and matured, fertilized in vitro and cultured (IVP) in medium supplemented with the following panel of embryo and / or endometrial -secreted cytokines and the pregnancy associated hormone progesterone (P4): Leukemia inhibitory factor (LIF), progesterone (P4), interferon gamma (IFNG), interleukin (IL) -1B, IL-3, IL-4, IL-10, granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor GM-CSF. These factors were chosen because of their previously reported roles in regulating *MHC-I* expression and/or embryo development and the establishment and maintenance of pregnancy. Cleavage rate and Day 7 blastocyst number were recorded during IVP. Day 7 blastocysts were snap frozen in pools of 10 and stored at –80^{o}C. Messenger RNA was extracted from replicate pools of embryos and processed for quantitative real time PCR to compare transcript abundance of non classical (NC) MHC-I genes (BOLA-NC1, NC2, NC3 & NC4). The data was analyzed using the standard curve method and means were compared by the student t–test. There was no effect of any of the cytokines tested on cleavage rate or blastocyst development. However, embryo mRNA expression of the the BOLA NC genes was significantly ($p \leq 0.05$) modified in a gene and cytokine specific manner. IL–3, P4, IFN-γ and IL–4 increased Day 7 NC1 mRNA expression, IL-4 significantly increased and IFNG significantly decreased NC2 expression, IL-3 significantly increased and IFNG significantly decreased NC3 expression and NC4 expression was significantly upregulated by IL-4, IL-10, LIF and G-CSF and down regulated by IFNG. In contrast to the lack of effect on in vitro blastocyst development, the NC MHC–I expression data suggests a preferential immunomodulatory role of these cytokines during preimplantation embryo development.

Poster 2

Ahmed Aldarmahi
Sarah Elliott, Carmen Almiñana, Alireza Fazeli
PhD Student
University of Sheffield, UK

A systemic approach to characterise and define an *in Vitro* model to Study the Female Reproductive tract Interaction with

The physiological interaction between gametes/embryo and female reproductive tract (FRT) involves intimate and precise processes to establish and maintain gamete/embryo development and pregnancy. The mechanisms underlying gamete and embryo interaction with maternal environment are not well understood. Several studies showed that the presence of spermatozoa, oocyte or embryo within FRT is altered the gene expression and proteomic profiles. Nevertheless, no established *in vitro* model currently exists to allow detailed and systemic investigation of maternal communications with gametes and embryo.

The aim of the current study is to characterise and define an *in vitro* model based on boar sperm interaction with a porcine telomerase-immortalised oviductal epithelial cell line (TERT-OVEC) to understand the nature of this process and evaluate different variables that may affect it in a systemic approach (system biology). These factors are mainly categorised into sperm factors and cell factors. Sperm factors include presence of sperm, sperm viability (live versus dead), concentration of sperm (10^3, 10^6 and 10^7 spermatozoa/ml) and source of spermatozoa. Cell factors include cell culture passage, type of cell (reproductive versus non-reproductive cell). We also co-cultured oviductal cells with inert substances (glass beads).

Presence of live spermatozoa, presence of dead spermatozoa and absent of spermatozoa (10^6 spermatozoa/ml) were co-cultured with a porcine oviductal cell line at 37°C, 5 % CO_2 for 24 hour. We have used three different sources of sperm for each group and repeated it in three consecutive passages (66, 67 and 68). Glass beads were co-cultured with oviductal cells (10^6 Beads/ml). Oviductal epithelial cell line without sperm was served as a control. Kidney epithelial cell line (LLCPK 1) was used in place of oviductal epithelial cell line and co-cultured with spermatozoa for the same conditions. RNA for each experiment was extracted, purified and cDNA was synthesised and used for quantitative real-time PCR. Three genes reported to be altered in oviduct in response to spermatozoa were selected. These genes are heat shock 70 kDa protein 8 (*HSPA8*), Adrenomedulin (*ADM*) and prostaglandin E Synthase (*PTGES*) and were considered to be the end point of our assay.

The results showed an alteration in gene expression in response to the presence of live spermatozoa. Different sources of boar semen were not as effective as live spermatozoa in inducing of oviductal transcriptome alterations. Different genes showed varied responses to increasing sperm concentrations. Interestingly, gene expression was generally down-regulated when the cell passage is increased. Data for glass beads and LLCPK1 cells are currently under analysis.

Current data indicated that different factors can influence the results of a defined *in vitro* model for investigation of maternal interactions with gametes and embryo. Further experiments are in progress to characterise other factors that can influence transcriptome changes in maternal tract in response to gametes and embryos.

Poster 3

Carmen Almiñana
Paul Heath, Ahmed Aldarmahi, Juan Maria Vazquez, Jordi Roca, Emilio Arsenio Martinez, Alireza Fazeli
Postdoctoral Research Associate
University of Sheffild, UK

A systems biology approach for understanding maternal communication with embryo

tAs the embryo migrates through the female reproductive tract, during the early stages of its development, a dialogue takes places between embryo and maternal tract. This cross talk is crucial in maintaining pregnancy and promoting the development of healthy offspring. Understanding the molecular mechanisms involved in this dialogue has practical implications in reproductive and developmental medicine. Here, we proposed a novel in vivo experimental model in porcine together with a high-throughput microarray analysis technique to identify the local factors involved in maternal communication with embryos. We hypothesized that the presence of pig embryo in the sow reproductive tract induces local response(s) in the uterine horn. To validate our hypothesis, each sow was subjected to laparoscopic insemination. While one oviduct was inseminated with spermatozoa the contralateral oviduct was inseminated only with diluent. Six days after laparoscopic inseminations, uterine horn flushings and endometrial samples were collected from both horns in each animal by hysterectomy. The presence of embryos at different embryo development stages in one horn and the existence of unfertilized oocytes in the other horn were verified by careful examination of uterine flushings. Microarray analysis was performed to depict differences in gene expression between groups using the Affymetrix Porcine Genome array. Thereafter, a specific altered gene expression was followed by qPCR at different stages of pregnancy.

Two hundred and eight transcripts were down-regulated in the presence of the blastocyst stage in the endometrium. Bioinformatics analysis using KEGG Pathways database classified the differentially expressed genes into four major categories, Metabolism (26%), Genetic Information Processing (19%), Environmental Information Processing (14%) and Cellular Processes (42%). The largest sub-pathways were involved in Signal Transduction, Cell Communication and with a major impact on Immune System. A deeper analysis was focused on one of those genes involved in Immune System pathways, specifically Toll Like Receptors pathway. Following TICAM2 gene expression in the present or absence of the embryo at different stages of pregnancy in uterine horn by qPCR, revealed changes in the endometrial transcriptome depending on the stages of pregnancy.
Further experiments are currently in progress to establish a mathematical model of TICAM2 gene expression to demonstrate regulatory factors involved in Immune System during early stages of pregnancy.

The genes identified here can be used to gain an overview of maternal Immune System response towards embryo. The integration of the local response described here with available biological data of local factors involved in embryo-maternal crosstalk could be a powerful systems biology approach for understanding maternal communications with embryo(s). This study was supported by grants from Seneca Foundation and MICINN.

Poster 4

Signe Altmäe[1]
Jüri Reimand[2], Triin Laisk[1], Merli Saare[3], Maire Peters[3], Jaak Vilo[2], Anneli Stavreus-Evers[4] and Andres Salumets[1, 3, 5]
PhD Student
University of Tartu, Competence Centre on Reproductive Medicine, Estonia

[1] Competence Centre on Reproductive Medicine and Biology, University of Tartu, Tartu, Estonia
[2] Department of Computer Science, University of Tartu, Estonia
[3] Department of Obstetrics and Gynaecology, University of Tartu, Estonia
[4] Department of Women's and Children's Health, Uppsala University, Sweden
[5] Department of Biotechnology, University of Tartu, Estonia

Embryo – endometrium interactions at the time of implantation

Background: Successful implantation depends on the complex interactions between the implanting embryo and the receptive endometrium. Numerous whole genome microarray analyses have identified hundreds of simultaneously up- and down-regulated endometrial genes that play role in the human receptive endometrium. The knowledge of the transcription profiles of human embryos is much more limited, only a few studies have been reported, and the understanding of the complex dialogue between the conceptus and the endometrium is scarce.

Objective: Our aim was to analyse and identify the possible interactions between the embryo and the endometrium at the time of implantation.

Methods: Affymetrix arrays HG-U133 Plus 2.0 were used for endometrial gene expression (mid-secretory phase endometrium n=4, proliferative phase endometrium n=4) and for embryonic gene expression analyses (30 8-cell embryos pooled n=2, 30 blastocyst stage embryos pooled n=2). Protein-protein interactions (PPI) from the Human Protein Reference Database (HPRD) were mapped to genes up-regulated in embryos (blastocyst *vs* 8-cell embryos) and genes up-regulated in endometrium (proliferative *vs* mid-secretory), followed by filtering using Gene Ontology cell compartment annotations. The resulting PPI network was clustered into overlapping modules with a novel greedy algorithm that accounts for statistical enrichments between proteins and their interaction partners.

Results and conclusion: In total we identified 442 interactions between studied genes, 91 interactions remained valid after the critical evaluation of the gene's function and it's location. A number of genes like LEP, LEPR, VCAN, TGFB1, laminins, collagens and fibulins, that are known to be involved in the implantation, were confirmed in our study. Meanwhile several new interactions were identified, where molecules APP, BMP2, DSC2, PDGFRA and ADAMST1 and others were involved. To our knowledge, we are the first to demonstrate the interaction pattern between the human implanting embryo and the endometrium.

Poster 5

Mark Burkitt
Dawn Walker, Daniela M. Romano, Alireza Fazeli
PhD Student
University of Sheffield, UK

Using Computational Systems Biology to Investigate Sperm Navigation and Transport in the Female Reproductive Tract

The mammalian oviduct is a complex environment, where the oviductal tube wraps around itself and other organs forming complex bends. This influences both the internal folds of soft tissue and the movement and interactions of gametes within. Several competing and complementary theories on how sperm navigate through the oviduct and find the oocyte have been proposed. However, many theories fail to fully consider the impact that the complex physical form of the oviduct may have on this movement.

The aim of this project is to create a computational model of sperm movement within the female reproductive tract to test the feasibility of several proposed hypothesises. These include sperm swimming without guidance, sperm guidance through chemotactic attraction to follicular fluid and thermotactic navigation due to temperature changes in the female reproductive tract.

We utilise computational techniques commonly used to model complex systems, such as agent based modelling, finite element modelling and image processing, all using high performance computing. The project is still ongoing, and can be split into several parts.

Firstly, two different types of 3D environment have been created. The first is a virtual reconstruction of a Zigmond chemotaxis chamber based on published dimensions. The second is a 3D representation of an oviduct. The mouse was chosen due to its size, making it relatively simple to capture information about the internal and external structure using histology images.

Sperm are autonomous individuals without intelligence, whose behaviour is entirely determined by interactions with their environment. Their behaviour is recreated by identifying a set of properties about how individual sperm behave under different conditions from appropriate literature, and encoded as parameters and rules. This process ensures that accurate spatial and temporal scales are used, and helps to ensure the behaviour and underlying processes are valid. This information is then used to simulate a large number of sperm using agent based modelling techniques.

The environmental conditions such as fluid viscosity, chemical composition and temperature still need to be created within the two types of 3D model to an appropriate level of detail. Creating an accurate fluid dynamics simulation within complex environments is not feasible due to limitations of computational power; therefore high level approximations will be used. Chemical and temperature diffusion will be simulated using finite element modelling. The conditions in both environments will be recreated to match those described in relevant literature.

Once the simulation components have been completed, a series of virtual experiments will be conducted. The initial set of experiments will be to ensure that the behaviour of sperm and the environmental conditions work correctly. A series of virtual experiments will then be performed to investigate different sperm navigation theories. For each theory, the behaviour of the agents will be measured within the chemotaxis chamber environment. The agents will then be migrated to the realistic 3D oviduct, and the behaviour compared. The validation of the model parameters and processes will be performed using measurements of individual sperm obtained from literature. Statistical validation of system level behaviour will also be performed.

Poster 6

Ido Carmel
S. Schnakenberg[2], M. Seigal[2], Y. Heifetz[1]
PhD Student
Hebrew University, Department of Entomology, Rehovot, Israel

1, Department of Entomology, Hebrew University, Israel;
2, Department of Biology, New York University, USA

miRNAs play a role in the regulation of the Drosophila female mating response

In Drosophila, like most insects, mating induces a rapid change in the female's physiology and behavior. In the hours after mating, major changes in gene expression and reproductive tract (RT) morphology occur. Immediately after mating, 79 genes in the lower RT exhibit a change in expression level, 70% of which are down-regulated. By 6 hours post-mating, major changes in expression profile of the female lower RT is evident. The rapid changes in mRNA and protein levels after mating, and in particular the lack of correlation between mRNA and protein levels for some genes, led us to hypothesize that the female response to mating involves post-transcriptional regulation by miRNAs. To test this hypothesis, we performed a bioinformatics analysis using expression profiling data of the lower RT of unmated and mated females. The analysis suggests that miRNAs negatively controls mRNA levels after mating in the lower RT, namely, there are miRNAs that are induced by mating. As an initial test for a possible role of *miRNAs* in reproduction, we reduced the function of all miRNAs by driving RNAi against *Dicer-1* and *Drosha* in the whole female body pre-mating. A clear reduction in female fecundity and fertility was observed in the first day after mating. We further dissected the effect of reduced function of all miRNAs in the lower RT, specifically in the Spermathecae Secretory Cells (SSC), both pre-mating and post-mating. At both examined timepoints, reduced function of all miRNAs in the SSC reduced female fecundity 6 hours post-mating but had no effect of female fertility. We thus conclude that reduced function of miRNAs in the SSC affects regulation of the initial activation of ovulation/egg-laying. Our results suggest a role for miRNAs in reproduction and more specifically in RT maturation and in the very early mating response.

Poster 7

Eva Correia
Muñoz, Marta; Díez, Carmen; Caamaño, Néstor; Gómez, Enrique
PhD Student
Centro de Biotecnología Animal – SERIDA; Área Genética y Reproducción Animal, Spain

Identification and functional analysis of specific proteins synthesized in the bovine uterus during the early development

Efficient embryo-maternal communication is critical for early embryonic development, implantation and pregnancy maintenance. Therefore, understanding molecular basis modulating such interactions could lead to an improvement of efficiency within reproductive biotechnologies.

The main objective of our project is the identification and functional analysis of maternal proteins with embryotrophic effect throughout bovine preimplantation development. We begun to analyze the uterine fluid (UF) recovered from recipients carrying embryos for 3 or 4 days in uterus during early stages, and compared the responses to those when such recipients contained no embryos (i.e. sham transferred). The initial analysis consisted of differential, two-dimensional gel electrophoresis coupled to mass spectrometry, a methodology allowing to detect up to ten thousand proteins. Preliminary results suggest that a number of responses would exist depending on the presence of embryos and probably their status (i.e., live, dead, lysed / non-recovered).

The origin of the proteins that can be present in UF recoveries (i.e. from either the maternal or embryonic side), will be elucidated by analyzing mRNA by RT-PCR and in-situ hybridisation in endometrial samples and recovered embryos. The cellular distribution of some selected, identified proteins will also be established by immunocytochemistry.

Finally, we will perform the functional analysis of the identified proteins by assaying their effects within *in vitro* embryo culture. We will evaluate development rates and quality of the blastocysts produced, by total and differential cell counts, apoptosis rate and cryotolerance.

Embryo-maternal interactions are based on complex networks whose study requires multiscale approaches. Hence it might be interesting to include in our project shotgun transcpriptomic and genomic techniques which will provide complementary data leading to a more robust experimental model with enhanced interest.

Poster 8

Anita Franczak
Bartosz Wojciechowicz, Genowefa Kotwica
Lecturer
Department of Animal Physiology, Faculty of Biology, University of Warmia and Mazury in Olsztyn, Poland

Endometrial and myometrial expression of aromatase P450 and the effect of IL1β and IL6 on estradiol 17β secretion in porcine uterine tissues during early pregnancy

The system of cytokines and steroids may influence the embryo-uterine interactions. Peri-implantation porcine embryos express interleukin 1β (IL1β) and interleukin 6 (IL6) that could affect conceptus-uterine communication and uterine activity during early pregnancy. Estradiol 17β (E_2) was found to provide a signal for maternal recognition of pregnancy in pigs. However, we have demonstrated previously active estrogen production by the endometrium and myometrium as an alternative source for this signal for recognition of pregnancy in the pig. We hypothesized that IL1β and IL6 can be involved in the regulation of E_2 secretion in the uterus during early pregnancy. We have examined: (1) the activity of aromatase cytochrome P450 (P450aro) in the endometrium and the myometrium harvested during days 10 to 11, 12 to 13 and 15 to 16 of pregnancy, and (2) secretion of E_2 by porcine uterine tissues in the presence of IL1β and IL6. The effects observed in pregnant pigs we compared with cyclic gilts at respective days of the estrous cycle. Individual myometrial and endometrial slices were placed in culture vials containing 2 ml medium M199, preincubated (18 h, 37^{o}C, 95% O_2 and 5% CO_2) and next incubated for 6 h and 12 h with control medium and cytokines: IL1β, IL6 (1 ng/ml and10 ng/ml, each). After incubation the culture medium was collected and the concentration of E_2 was determined. Expression of P450aro in uterine cross-sections was determined using immunofluorescency. We found that P450aro activity is statistically higher in pregnant endometrium than in myometrium. The highest activity of endometrial P450aro was observed during days 15 to 16 of pregnancy. In cyclic pigs P450aro activity did not differ. During days 10 to 11 of pregnancy endometrial secretion of E_2 was increased in the presence of IL1β (1 ng/ml) after 6 h of *in vitro* culture, while myometrial slices responded positively to IL6 (10 ng/ml) after 12 h of *in vitro* culture. On days 12 to 13 of pregnancy only endometrium responded to IL1β (10 ng/ml) positively. During days 15 to 16 of pregnancy both IL1β and IL6 enhanced secretion of E_2 in the uterine tissues. In cyclic pigs these stimulatory effects of selected cytokines were not observed. In summary: the activity of P450aro and the effects of IL1β and IL6 on E_2 secretion in porcine uterus depend on the presence of embryos in the uterus.

This research was supported by grant N N311 0685 33 (2007-2010) from the Ministry of Science and Higher Education, Poland and The European Union with The European Social Fund.

Poster 9

Yael Heifetz
Zelinger E., Apel I., Carmel I., Kapelnikov A. Keidar, T
Lecturer
Hebrew University, Israel

Making inroads into the *Drosophila* female reproductive system

Studies of fertilization across species suggest that mating sensitizes the female reproductive tract (RT) at the cellular and molecular level to ensure a successful reproductive event. We hypothesize that in *Drosophila melanogaster* mating triggers the final maturation of the female RT, switching it from an unmated to mated physiological state. To uncover the mechanisms that underlie mating-induced maturation, we conducted comparative microarray, proteomic, morphological and functional studies to characterize the RT of unmated and mated Drosophila females. We subdivided the RT into two functionally different regions: the upper-RT, consisting of oviducts and the lower-RT consisting of sperm storage organs and accessory glands. We found that mating elicits distinct molecular changes in the female RT as it transitions from an unmated to a mated physiological state. Each region of the RT appears to bear a unique molecular signature that reflects its specialized function. Our combined transcriptional and proteomic profiling show that some post-mating changes in expression may be mediated by translation initiation or by post-translational mechanisms. Moreover, mating induced distinct morphological changes in the epithelium, musculature, and innervation of the RT. Together, our results suggest that mating triggers molecular changes and active tissue remodeling in the female RT that mediate its progression to a mature functional state. Our long term goal is to achieve a systems level understanding of the mechanism that underlines the transition from unmated to a mated state and how miss-regulating contributes to infertility.

Poster 10

Kamil Krawczynski
Monika M Kaczmarek
PhD Student
Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn**,** Poland

Expression of key components of RISC required for biogenesis and performance of miRNA in the porcine endometrium during early embryo-maternal cross-talk.

MicroRNAs (miRNAs) are short ~22 nucleotides long endogenous RNAs, which play a crucial role in the regulation of gene expression in eukaryotes through sequence-specific targeting of the 3'- untranslated region of messenger RNAs, which results in translational repression. Dicer and Argonaute (Ago) 1 through 4 have been identified as key components of RISC (RNA induced silencing complex), required for biogenesis and performance of miRNAs.

Endometrium is a dynamic tissue that undergoes specific cyclic changes under the control of ovarian steroids. The implantation process is a crucial step during early pregnancy and involves a reciprocal interaction between the blastocyst and receptive uterus to ensure proper initiation of embryo attachment. Many factors are involved in embryo implantation through endocrine, paracrine, autocrine and juxtacrine modulators, but there is still a lack of comprehensive information about the nature of molecular mechanisms of gene expression directed by this factors.

Since there is some evidence supporting miRNA involvement in many physiological processes as well as immune responses, only few reports suggest their participation in mammalian reproduction. Our experimental approaches are focused on miRNA role during early embryo-maternal crosstalk in the porcine uterus. Thus, the first objective of our studies was the determination of the gene expression of Dicer and Ago 1, 2, 3 and 4 in the endometrium obtained from crossbred gilts on day 9, 12 or 15-16 (n=5-6) of the estrous cycle or pregnancy and the gene expression was assessed by the real-time PCR. Analysis showed a significant increase in Ago 2 level on day 12 of pregnancy in comparison to days 9 and 15-16 ($p<0.001$). Additionally, the expression of this gene was significantly higher on day 12 of pregnancy than in corresponding day of the estrous cycle ($p<0.001$). Ago 4 expression was affected only by the reproductive status of the animal and showed higher level on day 12 of pregnancy (vs. estrous cycle; $p<0.05$). The expression of Ago 4 did not change during the progress of pregnancy or estrous cycle. However, there was no change in the expression of the main enzyme of miRNA biogenesis, Dicer. There were no significant differences in Dicer level neither between the subsequent days of pregnancy and estrous cycle nor between the corresponding days of pregnancy as well as estrous cycle. Similarly, the expression of Ago 3 was not altered by the reproductive status. Determination of Ago 1 expression was not successful due to lack of the specific amplification product.

The embryo–maternal dialogue during implantation is comprised of complex molecular events involving intricate changes in the uterine gene expression. The enormous burst of the uterine gene expression starts when embryo sends the first signals to the mother, which in swine occurs approximately on 12 day of pregnancy. Interestingly, presented results give us the first information about possible fluctuations in processing of miRNA in the pig, since there was a significant increase of Ago 2 and Ago 4 expression on day 12 of pregnancy.

Poster 11

Emma Lucas
Watkins, A.J., Smith, S.J., Mayhew, T.M., Lewis, R.M. and Fleming, T.P.
Postdoctoral Research Associate
University of Southampton, UK

Does maternal diet during preimplantation development regulate maternal-embryonic communication of nutrient availability through placental development and function? A Systems Biology Approach

tThe long term influence of the maternal environment during embryonic and fetal development on offspring health and risk of disease is well-established. However, the precise mechanisms through which the maternal environment results in altered offspring phenotype are less understood. Using our well-characterised mouse model of maternal protein undernutrition, as well as introducing a protein overnutrition model, we are taking a systems biology approach to investigate the role of the placenta in communicating maternal environment to the developing embryo and fetus. Past data from our model implies that maternal communication of nutrient availability to the preimplantation embryo modifies the pattern of growth both pre- and postnatally, which in turn associates with adult cardiovascular disease risk.

By feeding pregnant mice either low (LPD) or high protein diet (HPD) for the entirety of gestation or just for the preimplantation period of development (3.5 days post-coitum; Emb-LPD and Emb-HPD), we are able to examine the response of the placenta by late gestation to discrete windows of altered maternal environment. Conceptuses were weighed and dissected and tissues have been stored for analysis.

At gestational day 17, a significant reduction in placental weight was seen in the conceptuses of low protein diet (LPD) fed dams. Furthermore, a significant reduction in the fetus: placental weight ratio was observed in LPD, Emb-LPD and HPD (all $p<0.05$), and a statistical tendency ($p=0.059$) towards reduction in the Emb-HPD group.

In order to determine the role of placenta in programming offspring phenotype within these treatment groups we are investigating placental phenotype and function by three approaches. Firstly, we are examining the structural composition of the placenta by histological and stereological approaches, designed to identify alterations in maternal and fetal contribution to the placenta, as well as the theoretical transport capacity of the placenta according to measurements of blood vessel surfaces. Secondly, we are investigating the transcriptional activity of the placenta by Affymetrix Exon 1.0 microarray and RTqPCR analysis, followed by confirmation of expression changes at the protein level by western blotting. Finally, we are measuring the capacity of the placenta to transport non-metabolisable amino acid and glucose analogues using radiolabelled tracers.

In combination, these studies will allow us first to identify phenotypic change in the placenta other than weight, then to correlate these with changes in relevant gene expression pathways and functional capacity of the placenta. This will allow us to test our hypothesis that suboptimal maternal diet especially during preimplantation development regulates through placental development and function the growth characteristics and disease risk of the next generation.

Poster 12

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The effect of trophoblast cells secretions on PGHS-2 mRNA expression and PGE2 release from luminal epithelial and stromal cells of the porcine endometrium in vitro

Prostaglandins (PGs) of endometrial origin are critical for the establishment of pregnancy in many species, including pigs. Inhibition of PGs synthesis results in pregnancy failure (Krealing et al., 1985). The first limiting enzyme in PGs production is prostaglandin endoperoxide synthase-2 (PGHS-2; also known as cyclooxygenase-2). This enzyme catalyses the conversion of arachidonic acid into prostaglandin H2, which in turn may be converted into luteotropic PGE2. Elevated secretion of endometrial PGE2 is important for corpus luteum protection during early pregnancy, but also for vascular function in the uterus. In pigs, expression of PGHS-2 increases after day 10 of pregnancy (Ashworth et al., 2006; Blitek et al., 2006) and coincides with rapid conceptus development and trophoblast attachment to the endometrial epithelium.

The studies were conducted to evaluate the effect of trophoblast cells secretions on PGHS-2 mRNA expression and PGE2 release from luminal epithelial (LE) and stromal (ST) cells of the pig endometrium. In the first part of the experiment, porcine conceptuses were collected on day 14 after insemination and trophoblast was separated from embryonic disc, cut into small pieces and passed through syringe needle to obtain cell suspension (according to Corps et al., 1990). Cells were plated in 6 cm plastic culture dishes in culture medium (phenol-free Medium 199 supplemented with 5% charcoal-stripped newborn calf serum and penicillin/streptomycin). Cells were cultured for 3 days, then fresh medium was added and culture was performed for 48 h. After that time, medium was collected and stored for further use. In a second part of the experiment, LE and ST cells of the endometrium were isolated (according to Blitek and Ziecik, 2004) and cultured separately in 6-well plastic culture plates (Nunc) or co-cultured in collagen-coated inserts in 6-well plates (BD Biosciences). In a co-culture model, ST were set up on the bottom of 6-well plates and LE were cultured on collagen-coated membranes inserted into each well. After reaching the confluency, LE and ST were treated with trophoblast cells medium (TrM) for 24 h. Then, culture medium was stored for EIA of PGE2. Cells were used for RNA isolation and real-time PCR analysis of PGHS-2.

In endometrial cells cultured separately, PGHS-2 mRNA was increased in LE in comparison to control value after incubation with TrM ($P<0.05$). No differences were observed in PGHS-2 level in ST cells. Both, LE and ST responded to TrM treatment with elevated secretion of PGE2 ($P<0.01$ and $P<0.05$, respectively). In the co-culture model of endometrial cells, PGHS-2 mRNA expression was increased in both cell types. Moreover, PGE2 release from LE and ST increased 3.5-fold and 2-fold ($P<0.05$), respectively.

Summarizing, trofoblast cells secretions increased PGHS-2 expression and PGE2 release in the porcine endometrial cells in vitro. Additionally, the effect of conceptus secretions on ST is mediated by LE cells. These results suggest, that conceptus may modulate endometrial PGs synthesis and secretion during peri-implantation period in the pig.

Supported by MSHE grant N31104732/2777

Poster 13

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Expression and localization of the chaperone protein MRJ in pig gametes and gonads

A group of specialized molecules, known as DnaJ proteins, plays a pivotal role in several cellular functions, promoting the folding and assembly of nascent proteins, their transport into cell organelles, and the formation of mature protein complexes. MRJ (Mammalian Relative of DnaJ) is a member of this family of chaperone proteins and has been shown to favor the remodeling of round spermatids to flagellate spermatozoa. Further evidence indicates the involvement of MRJ in chorioallantoic fusion and placenta development. However, only scattered information is available on the role of this protein in the female reproductive tract and female gametes and the data available are limited to murine and human species. Here we investigate the expression and localization of MRJ protein in pig spermatozoa, testis, cumulus oocyte complexes and ovaries.

To this purpose, we extracted RNA from testis, granulosa cells and from pools of 5 oocytes. cDNA was amplified by Reverse Transcription-PCR (RT-PCR) using primers specifically designed for MRJ, based on sequence data bank available. The amplified products were separated on a 2% TAE agarose gel, purified, sequenced and aligned using ClustalW. Isolated spermatozoa protein extracts were resolved by SDS PAGE, immunoblotted and stained with an antibody for murine MRJ. In order to localize this protein in porcine spermatozoa, immunocytochemical analysis was carried out on pig semen using the previous antibodies and a suitable fluorescent secondary antibody.

RT-PCR showed MRJ protein expression in pig testis, immature oocytes and granulosa cells. Comparison of the obtained pig cDNA sequence with databases revealed a degree of homology of 96%.with the human, 90% with the bovine and 86% with the mouse MRJ protein genes. Furthermore western blotting data demonstrated the presence of a porcine MRJ-like protein with a MW of 30KDa in mature spermatozoa protein extracts. Immunocytochemical results showed a specific localization of this protein on the acrosome surface, centrosomal region and tail principal piece of pig male gametes.

Altogether our findings indicate expression of MRJ in pig gametes as well as in granulosa cells, with a highly conserved nucleotide sequence within mammalian species. The protein shows a specific localization in spermatozoa, consistent with what previously described in the mouse and human. Further investigation of the temporal and spatial regulation of MRJ expression in pig will be important to reveal the putative role of chaperones in porcine reproductive functions.

Poster 14

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Characterisation of expression of Non-Classical MHC I antigens during mammalian post-implantation embryo development

During the initiation of pregnancy, a complex regulation of innate and adaptive immune responses occur at the maternal- fetal interface to promote a tolerogenic micro-environment. The major histocompatibility complex class I (*MHC-I*) region is known to be involved at a number of levels in the establishment and maintenance of pregnancy and recent discoveries have revealed that the non-classical (NC) MHC- I molecules in particular, are associated with successful reproduction in humans and mice. To date, information concerning the role of MHC-I NC genes in bovine embryo development is relatively limited. The aim of the current study was to characterise the mRNA expression profile of the NC- MHC I genes during the implantation period in bovine embryos. To this end, embryos were recovered from pregnant beef-cross heifers at days 16, 17, 20, 24 and 34 post artificial insemination. The embryonic disc and trophectoderm were isolated and mRNA was extracted, quantified and reverse transcribed. Following which, relative abundance of trophectodermal NC-MHC-I (BOLA- NC1, NC2, NC3 & NC4) mRNA expression was studied using quantitative real time PCR. Our results indicate that bovine embryo trophectoderm mRNA expression of NC MHC-I increases with embryo development. Significantly, in comparison to NC1, NC2 and NC3 mRNA expression, we observed a 10 fold increase in NC4 expression at each stage of embryo development. These results suggest that NC-MHC-I genes are also involved in establishing pregnancy in cattle and indicate a potential role for NC4 as a trophoblast-derived signal involved in protecting the embryo from rejection by the maternal immune system. In conclusion, our data implicates NC4 as likely embryo – derived candidate involved in maternal -fetal tolerance possibly by modulating uterine natural killer cell and dendritic cell function or proliferation.

Poster 15

Agnieszka Waclawik

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Abstract Title: Effect of prostaglandin E2 synthase and prostaglandin F2α synthase silencing on the expression of prostaglandin E2 receptor in endometrial cells

Prostaglandin (PG) $F_{2\alpha}$ and PGE_2 produced in the uterine endometrium play an important role in the regulation of the oestrous cycle and in the establishment of pregnancy in many domestic species, such as the pig. $PGF_{2\alpha}$ exerts luteolytic and PGE_2 luteotrophic effect. Endometrium secretes elevated PGE_2 levels into the uterine lumen and utero-ovarian circulation during maternal recognition of pregnancy in the pig. We have shown recently, that PGE_2 stimulates expression of cyclooxygenase-2, PGE_2 synthase (mPGES-1) and PGE_2 receptor (EP2) in the porcine endometrium and also the endometrial EP2 expression is increased in endometrium on days 11-12 of pregnancy in comparison with days 11-12 of the oestrous cycle. The aim of this present study was to determine the effect of mPGES-1 and PGF synthase (PGFS) silencing in porcine endometrial stromal cells.

Specific short interfering RNA (siRNAs) against PGFS and mPGES-1 were constructed and cloned into a commercial siRNA-expression vector (pSuper). Endometrial stromal cells isolated from uterus of gilts on day 11-12 of the oestrous cycle, were cultured to 70% confluency and transfected by Lipofectamine with pSuper vectors expressing siRNA against mPGES-1, or PGFS, or with negative control vectors (containing a siRNA of a gene not involved in PG synthesis or empty pSuper vector). Content of PGFS, mPGES-1 and EP2 mRNA was determined by Real-time PCR. Secretion of PGE_2 was examined by EIA.

Transfection of endometrial stromal cells with the mPGES-1/siRNA vector, resulted in a reduction of mPGES-1 mRNA content by 50% ($p<0.001$) and PGE_2 secretion by 40% in comparison with controls. Moreover, in mPGES-1-silenced cells content of EP2 mRNA was decreased by 60%, whereas PGFS mRNA expression was not altered. Transfection of endometrial stromal cells by the PGFS/siRNA vector knocked down PGFS mRNA by 58% when compared with the controls ($p<0.01$) but the expression of EP2 mRNA remained unaltered.

These results indicate an interdependence between the expression of mPGES-1 and EP2 in porcine endometrial cells. The presented data further confirm the hypothesis that mPGES-1 and EP2 are involved in a positive feedback loop of PGE2 in porcine endometrium.

Supported partly by SCSR grant N311319135 (years 2008-2012) and the basic grant of the Institute (IRZBZ PAN-2008-ZMDH). A.W. is funded by the Foundation for Polish Science

Poster 16

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Presence of P450 aromatase (P450arom) and production of 17β-estradiol (E_2) by porcine endometrium and myometrium during early pregnancy and the estrous cycle (days 10 to 11, 12 to 13 and 14 to 16)

In all steroidogenic tissues, levels of 17β-estradiol (E_2) depend mostly on activity of P450 aromatase, which is involved in final stages of its biosynthesis. In pigs, embryo-derived E_2 provides a signal for maternal recognition of pregnancy. Previously we have described, that the porcine endometrium and myometrium may synthesize and secrete E_2 during both early pregnancy and the estrous cycle acting as an additional source of this steroid especially at the time of decreased production of E_2 by embryo during peri-implantation period. In this study we have examined if E_2 secretion levels are parallel with amount of P450arom in porcine endometrium and myometrium during early pregnancy compared with respective days of the estrous cycle. Slices of endometrium and myometrium were isolated from uteri of post pubertal pigs on days 10 to 11, 12 to 13 and 14 to 16 of pregnancy and the estrous cycle. The isolated slices were pre-incubated in 2 ml of M199 medium for 18 hours in atmosphere of 95%O2+5%CO2 in 37ºC and then incubated for 6 hours. Concentration of E_2 in medium was determined with radioimmunoassay. Fragments of both tissues were used to establish the presence of P450arom with indirect immunofluorescence (using rabbit anti-rat cytochrome P450arom polyclonal antibody as primary and goat anti-rabbit IgG conjugated with biotin as secondary antibody). Cy3-conjugated streptavidin was used to visualize antibody complex. Preparations were examined under epifluorescent microscope. Pictures taken with magnification of 500× and areas of fluorescence were determined with graphic software. Endometrium and myometrium were comparable sources of E_2 during days 10 to 11 and 12 to 13 of pregnancy and the estrous cycle. During days 10 to 11 no statistical differences were observed between the same tissues in different studied periods. Higher ($p<0.05$) amounts of P450arom were observed in the endometrium in compare with the myometrium during days 10 to 11 of pregnancy and the estrous cycle. The myometrium of the cyclic gilts was higher source of the enzyme than of the pregnant ones. On days 12 to 13 of the estrous cycle endometrium and myometrium harvested during the estrous cycle were higher ($p<0.05$) source of P450arom than tissues isolated during pregnancy. The endometrium was higher than the myometrium ($p<0.05$) source of E_2 during days 14 to 16 of pregnancy and than the endometrium harvested during these days of the estrous cycle. These results correspond to the amounts of the P450arom, which were higher ($p=0.05$) in pregnant endometrium than in pregnant myometrium and in cyclic endometrium ($p<0.05$). The myometrium during pregnancy secreted lower amounts ($p<0.05$) of E_2 than during the estrous cycle. During the estrous cycle the myometrium was higher ($p<0.05$) source of E_2 than the endometrium. The embryo-maternal interaction determine endometrial synthesis and secretion of E_2 during peri-implantation period (days 14 to 16 of pregnancy). Uterine E_2 production may supplement decreased embryonic secretion of this steroid which occurs after day 14. of pregnancy.

Research supported by: Ministry of Science and Higher Education N N311 0685 33 (2007-2010) and The European Union within The European Social Fund

Poster 17

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The influence of interleukin-1β on synthesis and secretion of prostaglandins by corpora lutea in cyclic and pregnant pigs.

Corpus luteum (CL) is a transient endocrine gland composing of steroidogenic cells, fibroblasts, endothelial cells and immune cells. The past studies showed an important role of cytokines releasing by immunocompetent cells existing in CL on regulation of luteal cells function. Thus, the aim of the present study was to determine whether interleukin-1β (IL-1β) modulates synthesis and secretion of prostaglandins in porcine CL. Prostaglandin E_2 (PGE_2) acts as a luteotrophic factor and may support CL lifespan whereas prostaglandin $F_2\alpha$ ($PGF_2\alpha$) has an opposite effect and takes part in functional and structural luteolysis. We used pigs during days 10 to 11, 12 to 13 and 15 to 16 of the estrous cycle (n=12) or pregnancy (n=12). The isolated slices of CL (100 mg) were pre-incubated for 18 h in 2 ml of M199 medium with 0.1% BSA in atmosphere of 95% O_2 + 5% CO_2 in 37°C and then the medium was changed to fresh and tissues were treated with IL1β (at the dose of 10 ng/ml) and incubated for the next 12 hours. The culture medium was collected and the concentrations of PGE_2 was measured with ELISA. $PGF_2\alpha$ concentrations were tested with radioimmunoassay. The luteal expression of cycloxygenase-2 (*COX-2*), PGE_2 synthase (*mPGEs-1*) and $PGF_2\alpha$ synthase (*PGFs*) mRNAs were evaluated with Real-Time PCR. In results; the basal secretion of PGE_2 was higher ($p<0.05$) on days 15 to 16 of pregnancy than on corresponding days of the estrous cycle. The basic production of PGE_2 was decreased during studies days of the estrous cycle and was the lowest during days 15 to 16. Interleukin 1β increased PGE_2 ($p<0.05$) secretion from CL harvested on days 10 to 11, 15 to 16 of the estrous cycle and on days 10 to 11 of the pregnancy. The highest secretion of $PGF_2\alpha$ was observed on days 15-16 of the pregnancy and the estrous cycle. Luteal production of $PGF_2\alpha$ in response to IL -1β was the highest on days 10 to 11 of cycle. In the other studied periods of cycle and pregnancy, IL-1β did not influence on the $PGF_2\alpha$ secretion from CL. The luteal expression of *COX-2* of mRNA was stimulated by IL-1β in all studied days of pregnancy and the estrous cycle in pigs. The expression of mRNA *mPGEs-1* and *PGFs* in CL under the influence of IL-1β was not changed. In conclusion: IL -1β had a luteotrophic effect on pregnant and cyclic porcine CL by stimulation of secretion of PGE_2 and expression of mRNA *COX-2*.

Supported by grant KBN N N311 0685 33 (2007-2010) and the European Union within the European Social Fund

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ESF provides the COST Office through an EC Contract

COST is supported by the EU RTD Framework programme

Published by GEMINI COST ACTION FA0702

Book Title: Systems Biology in Maternal Communication With Gametes and Embryos

Year of Publication: 2010

ISBN: 978-0-9563694-3-7