

# Maternal communication with Gametes and Embryo



*A waterpaint of the view of Alghero - gently permission from Francesca Amalia Grimaldi*

***Proceeding of the 2<sup>nd</sup> General Meeting  
of GEMINI, Sardinia, Italy,  
1<sup>st</sup> - 3<sup>rd</sup> October 2009***

**Editors:  
Alireza Fazeli, Fulvio Gandolfi and Sergio Ledda**



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# EUROPEAN CO-OPERATION IN SCIENCE & TECHNOLOGY (COST)

COST - the acronym for European Cooperation in Science and Technology- is the oldest and widest European intergovernmental network for cooperation in research. Established by the Ministerial Conference in November 1971, COST is presently used by the scientific communities of 35 European countries to cooperate in common research projects supported by national funds. The funds provided by COST - less than 1% of the total value of the projects - support the COST cooperation networks (COST Actions) through which, with EUR 30 million per year, more than 30 000 European scientists are involved in research having a total value which exceeds EUR 2 billion per year. This is the financial worth of the European added value which COST achieves. A "bottom up approach" (the initiative of launching a COST Action comes from the European scientists themselves), "à la carte participation" (only countries interested in the Action participate), "equality of access" (participation is open also to the scientific communities of countries not belonging to the European Union) and "flexible structure" (easy implementation and light management of the research initiatives) are the main characteristics of COST. As precursor of advanced multidisciplinary research COST has a very important role for the realisation of the European Research Area (ERA) anticipating and complementing the activities of the Framework Programmes, constituting a "bridge" towards the scientific communities of emerging countries, increasing the mobility of researchers across Europe and fostering the establishment of "Networks of Excellence" in many key scientific domains such as: Biomedicine and Molecular Biosciences; Food and Agriculture; Forests, their Products and Services; Materials, Physical and Nanosciences; Chemistry and Molecular Sciences and Technologies; Earth System Science and Environmental Management; Information and Communication Technologies; Transport and Urban Development; Individuals, Societies, Cultures and Health. It covers basic and more applied research and also addresses issues of pre-normative nature or of societal importance.

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# WELCOME TO THE 2<sup>ND</sup> ANNUAL MEETING OF GEMINI

## Message from the Chairman

GEMINI in less than two years of its existence has gone from strength to strength. After the lovely meeting last year in Volos, today we have gathered in Alghero. We should also not forget the successful workshop in Lansk earlier this year. I think as a result of these meetings and other activities supported by our action, the GEMINI community is becoming much closer together. This is evidenced by publications arising from GEMINI too. Later this year we will have a special edition of Theriogenology published that is dedicated to the papers produced during the Lansk meeting. The current proceeding in your hands is published as a book and is deposited in reference libraries and can be downloaded from the GEMINI website.

These achievements are the results of 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, in Alghero plenty of time has been dedicated to discussion between each other. I believe this is quality time and we have to take advantage of it, to further develop research ties between ourselves.

Here I would like to particularly thank Sergio and Fulvio for their hard work in organising the current meeting in Alghero. Organising an international meeting is not an easy job. I am thankful to both of them for volunteering to organise this meeting and allowing us to visit the beautiful Alghero.

Another person, that without her help, we would not have been able to achieve half of what we have done in the last two years is the GEMINI administrator Mrs Sara Gottliebsen. I am thankful to her for the professional and accurate service that she is providing for the GEMINI community.

I want also to take this opportunity to thank all our invited speakers that have accepted our invitation to come to Alghero. 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.

Finally, I want to thank all of you for participating in this meeting. 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 programs that plays the major role in creating a success story of our COST ACTION.

I look forward to 3 days of discussion about Science, how to do Science, making new friends and creating partnerships.

Dr Alireza Fazeli

GEMINI Chairman  
September 2009



# WELCOME TO ALGHERO

Latitude 40° 33'0" N / Longitude 8° 19'0" E, this is exactly where we are. *Welcome to Alghero and welcome to Sardinia.*

It was a great pleasure for us that our proposal to organize the second COST-GEMINI General Meeting was accepted during the first Meeting in Volos, almost exactly one year ago. We hope that you will enjoy our hospitality despite the home made organization dictated by the usual budget restrictions.

The program deals with the interaction between gametes and reproductive tract, a very specific area of research explored by the stimulating contributions from scientists arriving from 20 different countries. Many of you are involved as speakers, presenters of oral communications or posters, all will attend the conference for meeting old and new colleagues. We wish to thank everybody and in particular those who arrive from the farthest countries.

We would like to acknowledge the great work done by all the young components of the local organising committee who smoothly switched from the preparation of the lunch menus to that of the culture media (hopefully without any confusion).

Finally a sincere thank goes to Professor Salvatore Naitana, Dean of Veterinary Faculty of the University of Sassari and Professor Antonio Leoni, Director the Department of Veterinary Pathology and Clinics for their generous support. A special thanks also to Professor Alessandro Maida the Rector of the University of Sassari for his constant attention to the meeting initiative. Last but not least our warm gratitude to the Fondazione Banco di Sardegna for the logistic support.

Best wishes

**Sergio Ledda & Fulvio Gandolfi**  
Local Conference Organisers



# 2<sup>ND</sup> ANNUAL GEMINI MEETING

ALGHERO, SARDINIA, ITALY

1<sup>st</sup> – 3<sup>rd</sup> October 2009

## Programme

### Thursday 1<sup>st</sup> October 2009

18:00 Registration Open and Reception

### Friday 2<sup>nd</sup> October 2009

08:30-09:00 Registration open

09:00-09:10 Conference Chairman Opening: **Sergio Ledda, Alghero Italy**

09:10-09:15 Gemini Chairman Opening: **Alireza Fazeli, University of Sheffield, UK**

09:15-10:05 Plenary Lecture

**Yuri Lazebnik, Cold Spring Harbor Laboratory, USA**

*Can a biologist fix a radio?*

### Morning session WGI: Experimental Models

10:05-10:10 Introduction

**Ann Van Soom, Ghent University, Belgium**

**Andras Dinnyes, Gödöllő, Hungary**

10:10-10:55 **Veronique Duranthon, INRA, France**

*Rabbit as a pre and post-implantation developmental model*

Short communication (selected from abstracts)

10:55-11:10 **Tom Stout, Utrecht University, Netherlands**

*The role of oxytocin and prostaglandin receptors in maternal recognition of pregnancy in the mare*

**11:10-11:35 Coffee Break (plus poster viewing)**

11:35-11:50 **Wedad Aboussahoud, University of Sheffield, UK**

*Activation of Toll-Like Receptor five decreases human trophoblast cells attachment to endometrial cells; the impact of infection on maternal communication with embryo*

11:50-12:35 **Heiner Niemann, Institute of Farm Animal Genetics, Mariensee, Germany**

*Unravelling the transcriptome of bovine preimplantation embryos derived *in vivo**

12:35-13:05 **Discussion Workshop WGI:**  
*About Radios, Rabbits and RNA*  
Moderators: Ann Van Soom, Ghent University, Belgium  
Andras Dinnyes, Gödöllő, Hungary

**13:05-14:30 Lunch and Poster Session**

Afternoon Session WGII: Technologies

14:30-14:35 Introduction  
**Eckhard Wolf, LMU Munich, Germany**  
**Jean-Pierre Ozil, INRA, France**

14:35-15:20 **Philippe Andrey, INRA, France**  
*How-to: A practical approach to the mathematical modeling of biological systems*

Short communication (selected from abstracts)

15:20-15:35 **Stefan Bauersachs, LMU Munich, Germany**  
*Analysis of the endometrium transcriptome using RNA seq*

15:35-15:50 **Jackson Kirkman-Brown, University of Birmingham, UK**  
*Modifications to sperm flagellar movement due to interaction with physiological female tract rheology*

**15:50-16:15: Tea Break (plus poster viewing)**

16:15-17:00 **Thomas Werner, Genomatix Software, Germany**  
*Next generation sequencing: Opportunities and challenges on the example of ChIP-seq application*

**17:00-17:30 Discussion workshop WGII:**  
*Technologies*  
Moderators: Eckhard Wolf, LMU Munich, Germany  
Jean-Pierre Ozil, INRA, France

**17:30-17:45 Tea Break (plus poster viewing)**

17:45-18:30 **Discussion Workshop:**  
*The year passed in COST; What was good? What was bad? And what we need to do for the next year?*  
Moderator: Fulvio Gandolfi, University of Milan, Italy

19:30 Dinner

## Saturday 3<sup>rd</sup> October 2009

08:30 Registration open

08:30-09:15 “Discussion on funding”

*Have we had any success to raise funds for research as a result of GEMINI?  
Last year experiences with EU and ESF?*

*Promoting GEMINI as a research topic in European frame works.*

Moderator: Alireza Fazeli, University of Sheffield, UK

09:15-10:05 **Mike White, University of Liverpool, UK**

*Spatial and temporal information coding by the NF- $\kappa$ B system*

### Morning session: WIHI: The Search for Mechanisms

10:05-10:10 Introduction

**Tom Fleming, University of Southampton, UK**  
**Pascale Chavatte-Palmer, INRA, France**

10:10–10:55 **Kevin Sinclair, University of Nottingham, UK**

*Developmental origins of health and disease: Maternal diet and epigenetic programming in the oocyte and pre-implantation embryo*

**10:55–11:20 Coffee break (plus poster viewing)**

Short communication (selected from abstracts)

11:20–11:35 **Adam Watkins, University of Southampton, UK**

*Elevated offspring growth induced by maternal protein undernutrition exclusive during preimplantation development is associated with altered signalling through the mammalian target of rapamycin (mTOR) pathway in mice*

11:35-11:50 **Anne Navarrete Santos, Martin Luther University, Germany**

*What can we learn about embryo-maternal interactions from diabetic animal models?*

11:50-12:05 **Pascale Chavatte-Palmer (on behalf of Oliver Picone), INRA, France**

*Influence of a Hyperlipidic Hypercholesterolemic diet on Folliculogenesis and Adipophilin expression in the preimplantation embryo in rabbits*

12:05-12:20 **Veerle Van Hoeck, University of Antwerp, Belgium**

*The consequence of hyperlipidemic serum during IVP culture on embryo quality*

12:20-12:35 **Judith Eckert, University of Southampton, UK**

*Acute maternal protein overnutrition alters embryonic cell-lineage allocation, signalling activity and biosynthesis levels*

*12:35–14:15 Lunch (plus poster viewing)*

14:15 – 14:45 **Discussion forum WGIII:**

*The Search for Mechanisms*

Moderators: Tom Fleming, University of Southampton, UK

Pascale Chavatte-Palmer, INRA, France

14:45-14:55: Meeting Closure and Concluding Remarks

**Alireza Fazeli, GEMINI Chairman**

*14:55–15:15 Tea break*

15:15-18:00 “MC Meeting”

*19:30 Gala Dinner*

**Sunday 4<sup>th</sup> October 2009**

One day Excursion

**INVITED  
SPEAKER  
ABSTRACTS**



Friday 2<sup>nd</sup> October 2009  
09:15-10:05

**Professor Yuri Lazebnik**

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

**Can a biologist fix a radio?**

By trying to answer the question posed in the title I will argue that the existing disparity between the effort of biological research and its meaningful outcome can be explained by a similar disparity between the complexity of the processes that we study and the tools with which most of us *communicate*. This argument will be intended to support the view that creating commonly accepted tools of communication that are sufficiently sophisticated to adequately reflect biological processes and, importantly, are useful to an average biologist not only can be of significant practical benefit for a particular field, but might change the very foundation of how biological research is organized.

Friday 2<sup>nd</sup> October 2009  
10:10-10:55

## Professor Veronique Durathon

INRA, UMR 1198 Biologie du Développement et Reproduction, F-78350 Jouy en Josas, France

### Rabbit as a pre and post-implantation developmental model

After having played a leading role nearly one century ago in the emergence of mammalian experimental biology and physiology, the rabbit species has been today largely if not totally replaced by the mouse as a model both in academic sciences and biomedical research. However, recent data from the literature testify that the rabbit is rapidly regaining interest as a developmental model for several key reasons.

While this species as a whole fulfills the main conditions usually required for an animal model in reproductive sciences -moderate size, short gestation period, easy follow up of offspring development, high number of oocytes and embryos available, easy in vitro manipulation of early embryos, precise timing of ovulation and easy morphological staging of the conceptus- its embryos started to receive more attention when it became obvious that as in almost all mammalian species but the mouse, the transcriptional activation of the embryonic genome is progressive and spans over several cell cycles providing a windows of opportunity to unravel the role of nucleo-cytoplasmic interactions at the very beginning of embryo development. Moreover the epigenetic modifications associated with the pre implantation period of development in the rabbit appears to differ from those established in the mouse. These have probably been considered too quickly as paradigmatic of the mammalian preimplantation development. Rabbit embryo also appears to be pertinent for the study of peri-implantation development because its embryonic morphology is archetypal for mammals and its delayed implantation when compared to rodents makes it more easily accessible to physiological manipulations. In addition the hemochorial structure of its placenta makes it a close model to human for questioning foeto-maternal interactions. Finally while the lagomorph order to which the rabbit *Oryctolagus cuniculus* species belongs is phylogenetically as distant to human as rodents, rabbit gene sequences appear to have evolved less rapidly than rodent ones, thus remaining more similar to human ones. This is confirmed by the genomic resources available today for the analysis of this model and that will be presented.

Friday 2<sup>nd</sup> October 2009  
11:50-12:35

## Professor Heiner Niemann

Institute of Farm Animal Genetics, Mariensee, Germany

### Unravelling the transcriptome of bovine preimplantation embryos derived *in vivo*

Bovine embryos can be generated by *in vitro* fertilization or somatic nuclear transfer; however, these embryos differ from their *in vivo* counterparts in many aspects and exhibit a higher proportion of developmental abnormalities. The advent of the cDNA array technology has for the first time made possible to compare the global transcription patterns of the ~23,000 genes of the mammalian genome. To obtain reliable data, it is mandatory to integrate the genomic information with advanced bioinformatic tools. The main challenge for genomic analysis of oocytes and embryos is the tiny amount of RNA (2-5 ng). Technology has been developed for non-biased amplification of these tiny amounts of RNA found in single embryos to the point that microarray analysis is possible (3-5 µg). Cross-species hybridization (bovine to human) has been used to reveal important features of bovine embryonic transcriptional activity.

The sequence of the bovine genome has recently become available and bovine genomic arrays can now be applied to determine the global mRNA expression profile during preimplantation development. Recently, we have determined for the first time the transcriptomes of bovine metaphase II oocytes and all stages of preimplantation embryos developing *in vivo* up to the blastocyst using the Affymetrix GeneChip® Bovine Genome Array which examines ~23,000 transcripts. The data show that bovine oocytes and embryos transcribed a significantly higher number of genes than somatic cells. Several hundred genes were transcribed well before the 8- and 16-cell stage, at which the major activation of the bovine genome expression occurs. Importantly, stage-specific expression patterns in 2-cell, 4-cell, 8-cell stages, morulae and blastocysts, were detected, indicating highly dynamic changes in the embryonic transcriptome and in groups of transiently active genes. Pathway analysis revealed >120 biochemical pathways that are operative in early preimplantation bovine development. Significant differences were observed between the mRNA expression profiles of *in vivo* and *in vitro* matured oocytes, highlighting the need to include *in vivo* derived oocytes/embryos in studies evaluating assisted reproductive techniques.

This dataset enables to compare expression patterns of bovine *in vitro* produced and/or cloned embryos with those of *in vivo* derived embryos and will thus provide a major step towards in unravelling the underlying mechanisms for abnormalities in development, including the high proportion of embryonic losses after normal fertilization, or the large offspring syndrome (LOS) observed after transfer of *in vitro* produced and/or cloned embryos.

#### Selected references

Adjaye J, Herwig R, Herrmann D, Wruck W, BenKahla A, Brink TC, Nowak M, Carnwath JW, Hultschig C, Niemann H, Lehrach H (2004). Cross-species hybridisation of human and bovine orthologous genes on high density cDNA microarrays. *BMC-Genomics* **5**, 83 ([www.biomedcentral.com/1471-2164/5/83](http://www.biomedcentral.com/1471-2164/5/83)).

Adjaye J, Herwig R, Brink TC, Herrmann D, Greber B, Sudheer S, Groth D, Carnwath JW, Lehrach H, Niemann H (2007). Conserved molecular portraits of bovine and human blastocysts as a consequence of the transition from maternal to embryonic control of gene expression. *Physiol. Genom.* **31**, 315-27.

Kues WA, Sudheer S, Herrmann D, Carnwath JW, Havlicek V, Besenfelder U, Lehrach H, Adjaye J, Niemann H (2008). Genome-wide expression profiling reveals distinct clusters of transcriptional regulation during bovine preimplantation development *in vivo*. *Proc Natl Acad Sci USA* **105**, 19768-19773.

Friday 2<sup>nd</sup> October 2009  
14:35-15:20

**Professor Philippe Andrey**  
INRA, France

**How-to: A practical approach to the mathematical modeling of biological systems**

The recent development of the systems biology approach in the study of genetic, metabolic, and signaling networks has put a large emphasis on the mathematical and computational modeling of biological systems. My talk is intended as a first and practical introduction to these modeling approaches. Based on several examples, I will review the diversity of modeling strategies, which allows for representations at various scales and resolutions of the interactions, of the temporal and spatial dynamics, and of the individual components of biological networks. The contribution of modeling and of model analysis tools to the understanding of the architecture and dynamics of these systems, in particular in the context of the present COST action, will be underlined. Similarly, issues and challenges, such as parameter estimation and model composition, that the large-scale systems biology approach represents for modeling, will also be discussed.

**Friday 2<sup>nd</sup> October 2009**  
**16:15-17:00**

**Professor Thomas Werner**

Genomatix Software, Germany

**Next generation sequencing: Opportunities and challenges on the example of ChIP-seq application**

High through-put analysis has evolved from the large-scale sampling of existing knowledge (microarrays) to a truly exploratory stage with Next Generation Sequencing (NGS), which reveals new findings independent of prior knowledge. The advantages of NGS will be illustrated on an example of ChIP-seq: a genome-wide binding study of STAT1. After initial analysis, redefinition of the binding pattern is possible as well as a deeper analysis revealing different biological processes identified via functional context analysis of STAT1 binding.

Saturday 3<sup>rd</sup> October 2009  
09:15-10:05

**Professor Mike White**

University of Liverpool, United Kingdom

### **Spatial and temporal information coding by the *NF-κB* system**

The Nuclear Factor kappa B (NF-κB) transcription factor regulates cellular stress responses and the immune response to infection. NF-κB activation results in oscillations in nuclear NF-κB abundance (1). To define the function of these oscillations, we treated cells with repeated short pulses of tumor necrosis factor alpha (TNFα) at various intervals to mimic pulsatile inflammatory signals. At all pulse intervals analyzed, we observed synchronous cycles of NF-κB nuclear translocation. Lower frequency stimulations gave repeated full-amplitude translocations, whereas higher frequency pulses, gave reduced translocation, indicating a failure to reset. Deterministic and stochastic mathematical models predicted how negative feedback loops regulate both the resetting of the system and cellular heterogeneity. Altering the stimulation intervals gave different patterns of NF-κB-dependent gene expression, supporting a functional role for oscillation frequency (2). This system is one of a number of biological cycles that have been discovered. Other examples include calcium signalling, transcription cycles, p53, the segmentation clock, the circadian clock, the cell cycle and seasonal rhythms. Are such cycles a fundamental theme in the integration of biological systems? This could provide a mechanism to explain the robustness of cellular decision making processes.

(1). D.E. Nelson, A.E.C. Ihekweaba, M. Elliott, J. Johnson, C.A. Gibney, B.E. Foreman, G. Nelson, V. See, C.A. Horton, D.G. Spiller, S.W.

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Saturday 3<sup>rd</sup> October 2009  
10:10-10:55

**Professor Kevin Sinclair**  
University of Nottingham, United Kingdom

**Developmental origins of health and disease: Maternal diet and epigenetic programming in the oocyte and pre-implantation embryo**

The concept that certain adult diseases, such as hypertension, type 2 diabetes and dyslipidaemia, can originate from events occurring *in utero* arose from epidemiological studies in humans but has since been supported by numerous animal-based studies, the vast majority of which have been conducted in the sheep and rat where the effects of global nutrient restriction and low protein diets have been investigated. The long-term programming effects of specific dietary nutrients on key epigenetic processes during gametogenesis and pre-implantation development have been the focus of recent investigations at Nottingham. In the sheep we have demonstrated that physiologically relevant reductions in the dietary supply of specific B-vitamins (i.e. vitamin B<sub>12</sub>, folate) and methionine to intending mothers can epigenetically modify DNA in their progeny and lead to adult offspring with elevated blood pressure and early indicators of 'metabolic syndrome', effects most pronounced in male offspring [Sinclair et al. 2007. PNAS 104: 19352]. Parallel studies in the rat reveal common phenotypic effects which are also male specific. These studies set a precedent for the long-term programming effects of specific micronutrients during gametogenesis and pre-implantation development, emphasising the need for more basic research into these phenomena so that appropriate nutritional advice can be given to intending mothers and modifications made to existing empirically derived oocyte and embryo culture media.



**ORAL  
PRESENTATIONS  
FROM ABSTRACTS**



Friday 2<sup>nd</sup> October 2009

10:55-11:10

**Professor Tom Stout**

Professor

Utrecht University, Netherlands

Other Authors: Marta Villani

### **The role of oxytocin and prostaglandin receptors in maternal recognition of pregnancy in the mare**

Luteolysis in the mare is effected by  $\text{PGF}_{2\alpha}$  released by the endometrium during days 10-16 after ovulation in response to oxytocin (OT). While maternal recognition of pregnancy (MRP) in this species is known to involve a conceptus-directed suppression of OT-induced endometrial  $\text{PGF}_{2\alpha}$  release, it is not known how this suppression is orchestrated. We investigated aspects of MRP by examining how endometrial expression of the genes for OT and  $\text{PGF}_{2\alpha}$  receptors (OTR and FP), cyclo-oxygenase-2 (COX2) and a prostaglandin transporter (PGT) differed between cycling and early pregnant mares. Endometrial biopsies were recovered from 4 mares at each of late oestrus, days 7 and 14 of dioestrus, and days 7, 14 and 21 of pregnancy. Expression of mRNA for OTR, FP, COX2 and PGT was analyzed by quantitative rtPCR.

COX2 gene expression was lower on day 14 of pregnancy than dioestrus, suggesting that direct suppression of uterine prostaglandin synthesis contributes to initial abrogation of  $\text{PGF}_{2\alpha}$  release. However, by day 21 of pregnancy gene expression for both COX2 and OTR had increased significantly, indicating that prevention of luteolysis after conceptus fixation is due neither to reduced PG synthetic capacity nor to an absolute reduction in endometrial OT sensitivity. By contrast, FP gene expression was suppressed on days 14 and 21 of pregnancy, and we propose that reduced endometrial sensitivity to  $\text{PGF}_{2\alpha}$  plays an important role in MRP by uncoupling positive-feedback loops in which  $\text{PGF}_{2\alpha}$  would otherwise induce further release of both itself and OT from the endometrium. In short, while a reduction in OT-sensitivity and  $\text{PGF}_{2\alpha}$  production capacity may contribute to the antiluteolytic mechanism during the conceptus mobile phase, a reduction in  $\text{PGF}_{2\alpha}$  sensitivity is likely to be more important to sustained MRP by disabling the positive feedback loops required to generate the large pulses of  $\text{PGF}_{2\alpha}$  required to complete luteolysis.

**Friday 2<sup>nd</sup> October 2009**

11:35-11:50

**Mrs Wedad Aboussahoud**

PhD Student

University of Sheffield, UK

Other Authors: Bruce C, Fazeli A

**Activation of Toll-Like Receptor five decreases human trophoblast cells attachment to endometrial cells; the impact of infection on maternal communication with embryo**

Embryo implantation in to the uterine epithelium is a key process in establishing successful pregnancy. Implantation involves apposition and adhesion of the trophoblast cells to the epithelium lining of the endometrium. Ethical concerns regarding experimentation with primary human tissue during this period of life, necessitate creation of in-vitro models for understanding the basic mechanisms involved. Toll-Like receptors (TLRs) showed to play a major role in defence against pathogens invading the female reproductive tract (FRT). Here we report creation of a new in-vitro model for studying human endometrial embryonic interactions and the impact of activation of TLR5 on attachment of trophoblast cells to the endometrial cells. An in vitro assay using Human monolayer-cultured, telomerase immortalised uterine epithelial cell line (hTERT-EECs) and Jar cells (choriocarcinoma human trophoblast cells) was used to investigate attachment of human embryo to endometrium. JAR spheroids attached and adhered to hTERT-EECs in a time and concentration dependent manner. Application of differentiation factors such as Methotrexate and Phorbol 12-Myristate 13-Acetate inversely affected the number of spheroid binding to endometrial cells. Flagellin was used as a pathogen associated molecular pattern (PAMPs) to simulate the effect of infection on maternal interaction with embryo. Our results showed that presence of PAMPs impacted the embryo attachment to the endometrium through TLR5 activation. These findings may demonstrate the underlying pathology in cases such as recurrent miscarriage and IVF implantation failure. These data may provide new opportunities in management of infertility cases in the clinic.

**Friday 2<sup>nd</sup> October 2009**

15:20-15:35

**Dr Stefan Bauersachs**

Junior Group Leader

LMU Munich, Germany

Other Authors: Alexander Graf, Karin Gross, Sebastian Eck, Anna Benet-Pages, Tim Strom,  
Eckhard Wolf

**Analysis of the endometrium transcriptome using RNAseq**

Next-generation sequencing technologies open up new dimensions of transcriptome analyses. Deep sequencing allows identification of nearly all existing poly(A) RNA sequences in a cell or tissue with ultra-high sensitivity below one mRNA copy per cell. Digital gene expression analysis by counting RNA sequence reads circumvents problems with data processing as compared to microarray analyses. Particularly for organisms with incomplete gene annotations RNAseq overcomes restrictions of available microarrays that depend on the content of sequence databases. RNAseq data contain information beyond simple comparative expression analyses by providing more quantitative expression data and by identification of transcript variants such as splicing variants and 3' UTR variants. In a first experiment endometrial tissue samples from day 18 pregnant heifers (n=3) and corresponding nonpregnant control heifers (n=4) were used for preparation of RNAseq libraries. Paired-end 36 bp reads were produced on an Illumina Genome Analyzer II sequencing one sample per lane. This single run resulted in 63 million mappable paired-end reads. Based on the UMD2 assembly and annotation of the bovine genome sequence ~65% of the unique reads mapped to known exons, ~30% to intronic regions, and ~5% to intergenic regions. Different algorithms were applied to map exon junctions and 3' UTR regions and results were compared with existing gene annotations. Furthermore, RNAseq data were compared to microarray data obtained from the analysis of similar endometrial samples using the Affymetrix Bovine Genome Array. In conclusion, transcriptome analyses by high-throughput sequencing can provide important additional information on the regulation of mRNA expression, e.g. identification of as yet unknown transcripts, differential splicing and differential 3' UTR regions.

Friday 2<sup>nd</sup> October 2009

15:35-15:50

**Dr Jackson Kirkman-Brown**

Senior Lecturer

University of Birmingham, UK

Other Authors: D. J. Smith, E. A. Gaffney, H. Gadelha, N. Kapur

### **Modifications to sperm flagellar movement due to interaction with physiological female tract rheology**

The highly viscous and elastic liquids that line the female reproductive tract are crucially important but often over-looked features of sperm motility regulation. Investigation of sperm modulation by chemoattractants and clinical work-up of semen samples typically employs preparation into low viscosity medium. However the observed flagellar beat patterns, resulting motility and apparent activation state are significantly modified in these liquid media that have non-physiological rheology. To understand observations in any conditions the underlying fluid dynamics become crucial. We have already used these to explain how in normal culture media sperm become 'attracted' to boundaries.

We present high-speed flagellar beat acquisition of human sperm migrating in methylcellulose-containing medium that approximates the conditions in the human cervix. The flagellar beat is characterised in detail and compared with data for sperm in low viscosity laboratory medium.

We find that the approximately linear relationships between wavespeed / wavelength / beat frequency versus progressive velocity in low viscosity liquids are abolished at physiological (high) viscosities. This may occur because the sperm flagellum is subject to a limiting wavespeed in high viscosity liquid, which results in a trade-off between the other parameters through the relation wavespeed = frequency x wavelength. We also find that beat planarity is greatly increased in high viscosity liquid, and that the high viscosity waveform is characterised by increasing mean curvature from midpiece to the flagellar tip, with a relatively sharp increase from 20 to 27 microns from the head/midpiece junction.

The high viscosity waveform is easier to image accurately than the low viscosity waveform due to its greater planarity and slower wave speed. Incredibly for the sperm that can migrate in these viscosities, their speed of migration (progressive velocity) is not diminished! To hypothesise accurately any *in vivo* mechanism, future studies of motility-modulation should employ physiological viscosity liquids.

Saturday 3<sup>rd</sup> October 2009

11:20-11:35

**Dr Adam Watkins**

Postdoctoral Research Associate

University of Southampton, UK

Other Authors: E.S. Lucas, A. Wilkins, C. Osmond, J.J. Eckert, F.R.A. Cagampang, M.A. Hanson and T.P. Fleming

**Offspring growth, cardiovascular and adipose phenotypes display differential sensitivity to maternal protein under nutrition at one year of age in mice**

The majority of animal studies examining the long-term effects of offspring health following a maternal dietary challenge are typically terminated during young adulthood. Consequently, it remains unclear whether identified phenotypes persist into late adulthood. Here, we determine the stability of our initial offspring observations, made at 6 months of age, into mature adults (1 year).

Offspring from mothers fed normal protein diet (NPD; 18% casein) or isocaloric low protein diet (LPD; 9% casein) during oocyte maturation (termed Egg-NPD and Egg-LPD respectively), LPD exclusively during preimplantation development (termed Emb-LPD) or NPD or LPD throughout gestation (termed NPD and LPD respectively) were allowed to develop till 52 weeks of age. Weekly body weight measurements; systolic blood pressure (SBP) and organ allometry at 52 weeks as well as qRT-PCR determination of mRNA expression of uncoupling protein 1 (*Ucp1*), adrenergic receptor beta 3 (*Adrb3*), insulin receptor (*Insr*), and insulin-like growth factor I receptor (*Igf1r*) in interscapular and retro-kidney fat were made.

Emb-LPD and LPD females displayed significantly increased and decreased body weight profiles respectively when compared to NPD females. Egg-LPD, LPD and Emb-LPD offspring had significantly elevated SBP when compared to respective controls at 52 weeks. LPD females had significantly reduced inguinal and retro-kidney fat pad:body weight ratios when compared to controls. LPD females displayed significantly increased *Ucp1* and *Adrb3* expression in interscapular fat, whilst Emb-LPD females had increased *Igf1r* and *Insr* expression in retro-kidney fat when compared to controls.

These data show that maternal undernutrition during specific windows of the reproductive cycle, or development, induce long-term changes within the offspring for up to 1 year of age. Whilst all dietary challenges elevated offspring SBP, altered postnatal growth, organ allometry and fat gene expression only occurred when maternal LPD was administered post fertilisation, indicating window-specific sensitivity with respect to long-term offspring health and physiology.

**Saturday 3<sup>rd</sup> October 2009**

11:35-11:50

**Dr Anne Navarrete Santos**

Lecturer

Martin Luther University, Germany

Other Authors: René Thieme<sup>1</sup>, Nicole Ramin<sup>1,2</sup>, Sünje Fischer<sup>1</sup>, Maria Schindler<sup>1</sup> & Bernd Fischer<sup>1</sup>

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## **What can we learn about embryo-maternal interactions from diabetic animal models?**

Women with diabetes mellitus are subfertile. In humans, type 1 or insulin-dependent diabetes has been found to negatively affect pregnancy by causing poor prenatal outcomes and early miscarriage. The objective of this study was the establishment of a rabbit model for diabetes type 1 to investigate diabetogenous effects on maternal environment and blastocyst development during the preimplantation period.

Chemical agents like alloxan or streptozotocin destroy specifically the beta-cells of pancreatic Langerhans island. The alloxan-treatment results in a complete loss of endogenous insulin (hypoinsulinaemia) followed by elevated blood glucose levels about 14mmol/L (hyperglycaemia) defining a diabetes type 1. In female rabbits acute hyperglycemia was induced by a single alloxan treatment 10 days before mating. 48h after alloxan treatment the rabbits developed a diabetic metabolism. The circulating insulin level decreased (6.5fold) to approx. 15pM and the blood glucose levels increased to up to 20mM.

The fertility rate of the diabetic rabbits clearly mirrored subfertility. The blastocysts from diabetic females were characterized for embryonic disk development and early gastrulation stages at day 6 p.c. We found that the onset of gastrulation and Brachyury levels were delayed in diabetic blastocysts. In the uteri and blastocysts from diabetic females the expression pattern of the insulin-IGF1 system (IGF1, 2, insulin receptor, IGF1 and IGF2 receptor) and their metabolic target genes (hexokinase, phosphoenolpyruvat carboxykinase, glucose transporter 4) was distinctly different compared with controls. Furthermore the amount of apoptotic cells was increased in the embryonic disc from blastocysts grown in diabetic environment, correlating closely with the downregulation of the Bcl-X gene.

Taken together we demonstrate significant changes in the maternal environment and in blastocyst development as a result of diabetic conditions during pregnancy and a disruption of the insulin-dependent glucose metabolism. The dysfunction of the embryonic glucose metabolism may potentially be involved in the mechanism of diabetogenous embryopathies.

*Supported by the German Research Council (DFG; NA 418/4-2)*

**Saturday 3<sup>rd</sup> October 2009**

11:50-12:05

**Professor Pascale Chavatte-Palmer**

**On behalf of Mr Oliver Picone**

PhD Student

INRA, France

Other Authors: Cordier AG, Peynot N, Laigre P, Dahirel M, Larcher T, Chavatte-Palmer P, Duranthon V

**Influence of a Hyperlipidic Hypercholesterolemic diet on  
Folliculogenesis and Adipophilin expression in the  
preimplantation embryo in rabbits**

The administration of an hypercholesterolemic Hyperlipidic (HH) diet to female rabbits from 10 weeks of age decreases fertility and induces intra uterine growth restriction and subsequent obesity in the offspring. The aim of this study was to explore the origin of the decreased fertility through the evaluation of ovarian follicular maturation prior to conception and early embryo genomic expression thereafter.

First experiment: Female rabbits were fed with a control (2% fatty acids) or HH (8% soy oil and 0.2 % cholesterol) diet from 10 weeks of age. The ovaries were collected at 18 weeks, one week after synchronization with light stimulation (16H light/ 8H night). Follicle distribution and maturation stages were analysed by histology.

Second experiment: Using the same protocol, 16-24 cells embryos (48 hours post coitum, embryonic genome activation stage) were collected at 18 weeks of age. RNA were extracted from 4 independent pools of 20 embryos from HH diet and control does. Each RNA sample was subsequently amplified by two independent procedures (global RT-PCR or aRNA in vitro transcription), resulting in 16 hybridizations with a dedicated, custom-made micro-array. Results of hybridization were analysed by Anova (GENANOVA software).

**Results:** Histological analysis demonstrated a significantly lower number of antral follicles on the ovaries of HH does ( $p < 0.001$ ) and a significantly higher number of atretic follicles ( $p < 0.05$ ).

Ten genes were differentially expressed between both groups. Among these, adipophilin plays an important role fatty acid capture and storage. Overexpression of adipophilin in HH embryos was confirmed by qRT-PCR.

**Conclusion:** The HH diet affects both follicular development prior to conception and early embryo gene expression. These phenomena may play a role in the observed effects on fertility and offspring development. Endocrine profiles in females and gene expression in the oocyte and at the blastocyst stage are currently being explored.

**Saturday 3<sup>rd</sup> October 2009**

12:05-12:20

**Miss Veerle Van Hoeck**

PhD Student

University of Antwerp, Belgium

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<sup>3</sup>Department of Reproduction, Fertility and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium.

**The consequence of hyperlipidemic serum  
during IVP culture on embryo quality**

Obesity is frequently associated with female reproductive abnormalities. Hyperlipidemia might alter the oviductal micro-environment and potentially results in disappointing fertility outcome. Therefore, we investigated the consequences of nutritionally induced hyperlipidemic culture conditions on bovine in vitro embryo development and quality. Bovine zygotes were cultured in SOF medium supplemented with serum from heifers fed three successive dietary treatments: Control serum after a hay based diet, hyperlipidemic serum (FatCh) after a carbohydrate and protected palm oil rich diet, or hyperlipidemic serum (Fat) after a protected palm oil rich diet. Blastocysts were evaluated for developmental competence, total cell count, picnotic and mitotic indexes, and cryotolerance. Studied mRNA transcripts were quantified by quantitative RT-PCR. FatCh and Fat serum resulted in doubled total cholesterol ( $167.1 \pm 11.9$  and  $150.0 \pm 12.8$ , respectively versus  $83.4 \pm 13.7$  mg/dl) and fatty acid concentration ( $8146.60 \pm 214.61$  and  $6935.56 \pm 1081.04$ , respectively versus  $3944.0 \pm 425.07$   $\mu$ mol/l) compared to control serum. Supplementation of FatCh and Fat serum significantly reduced blastocyst rates (27.8 and 23.4, respectively versus 36.2%), total cell number ( $103.3 \pm 30.1$  and  $95.6 \pm 28.2$ , respectively versus  $146.9 \pm 34.2$ ), mitotic index ( $1.3 \pm 1.1$  and  $1.7 \pm 2.4$ , respectively versus  $3.6 \pm 2.2\%$ ) and hatching rates after vitrification (20.4 and 13.8, respectively versus 35.7%) compared to Control serum. FatCh and Fat embryos revealed a significantly higher relative transcript abundance of genes related to apoptosis and metabolism compared to Control embryos. This combined in vivo and in vitro model indicates that exposure of pre-implantation embryos to hyperlipidemic conditions may result in reduced embryo development and quality, hence poorer fertility.

*Support by the BBSRC, University of Southampton School of Medicine and Wellbeing of Women is gratefully acknowledged.*

1. Watkins AJ et al., *Biol Reprod.* 78:299-306, 2008. 2. Philp LK et al. *Am J Physiol Regul Integr Comp Physiol.* 295:R1982-90, 2008. 3. Zhu MJ et al., *Biol Reprod.* 71:1968-73, 2004.

Saturday 3<sup>rd</sup> October 2009

12:20-12:35

**Dr Judith Eckert**

Senior Lecturer

University of Southampton, UK

Other Authors: F. Lock, S. Haque, G. Premkumar, Y. Cheong, I.T. Cameron, T.P. Fleming  
& J.J. Eckert

**Acute maternal protein overnutrition alters embryonic cell-lineage allocation, signalling activity and biosynthesis levels**

Maternal malnutrition during fetal development alters metabolic processes involved in growth regulation of the developing fetus resulting in offspring obesity and metabolic diseases. Such phenotypic changes can be mediated by mechanisms for sensing energy status and adapting biosynthesis levels such as mTOR or AMPK (2,3). We have already established that maternal protein undernutrition *exclusively during the preimplantation period* can induce similar postnatal phenotypes (1). Measurable changes are detectable by the blastocyst stage when cell lineage divergence into inner cell mass (ICM, embryo proper) and trophoblast (TE, placental lineages) emerges, coinciding with major metabolic transitions. For example, blastocyst cell lineage allocation and mTOR signalling are altered, collectively suggesting compensatory mechanisms whereby energy status and signalling responses to nutrition maintain anabolic activity and growth potential of the conceptus. However, we know little about *directionality* of the responses to nutrition levels. Here, we compare cell allocation, protein biosynthesis and energy/stress status indicated by AMPK $\beta$ 1 distribution in blastocysts derived from mothers fed a control (18% casein) or elevated (30% casein, HPD) protein diet during the preimplantation period. In HPD-blastocysts, the ICM was reduced ( $p < 0.001$ ) whilst TE and total cells were maintained thus directing cells towards TE ( $p < 0.001$ ) just prior to implantation (d3.75). Such blastocysts synthesised more protein compared to controls (50% upregulation;  $p < 0.001$ ) up to 12hrs after removal from the challenge. AMPK $\beta$ 1 as indicator of cellular stress was found increased in TE nuclei in HPD blastocysts. Altogether, this suggests that early embryos can raise their anabolic metabolism in plentiful conditions. However, maternal protein overnutrition may cause cellular stress diverting blastomeres away from the ICM lineage towards the metabolically more active TE just prior to implantation. Such lineage diversion may help the embryo cope better with adverse intrauterine environments by tightly controlling the ICM environment and ensuring embryonic-maternal cross-talk sufficient for implantation.

*Support by the BBSRC, University of Southampton School of Medicine and Wellbeing of Women is gratefully acknowledged.*

1. Watkins AJ et al., *Biol Reprod.* 78:299-306, 2008. 2. Philp LK et al. *Am J Physiol Regul Integr Comp Physiol.* 295:R1982-90, 2008. 3. Zhu MJ et al., *Biol Reprod.* 71:1968-73, 2004.



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**POSTER  
PRESENTATIONS  
FROM ABSTRACTS**



**Dr Daniela Bebbere**

Federica Ariu<sup>1</sup>, Luisa Bogliolo<sup>1</sup>, Stefano Fois<sup>1</sup>, Stefano Nieddu<sup>1</sup>, Stefania Uccheddu<sup>1</sup>, Giovanni Giuseppe Leoni<sup>2</sup>, Sergio Ledda<sup>1</sup>  
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### **Messenger RNA expression patterns of Interferon-tau, OCT 4 and Nanog in ovine preimplantation embryos derived from different origins**

The objective of this study was to compare the relative abundance of important developmentally genes Interferon-tau (IFN-tau), OCT 4 and Nanog in ovine blastocysts derived from different origins. For this purpose, cumulus oocyte complexes derived from ovaries of regularly slaughtered one-month old prepubertal and adult sheep were matured (IVM), fertilized and cultured in vitro in standard conditions up to blastocysts stage. Parthenogenetic blastocysts were obtained after activation of in IVM adult oocytes with ionomycin and 6 dimetil amino purine (6-DMAP).

Three groups of 10 blastocysts for each class were used to quantify the relative expression of IFN-tau (as relevant expression of trophoblastic cells) and OCT4 and Nanog (mainly involved in ICM cell self-renewal). Analysis was performed by reverse transcription followed by Real Time PCR. The relative quantification of the target genes was calculated with the 2-ddCt method (Livak and Schmittgen, 2001 Methods 25: 402-8) after normalization against the  $\beta$ -actin expression levels.

Higher relative abundance for IFN-tau mRNA (ANOVA;  $P < 0.01$ ) was observed in prepubertal and parthenogenetic blastocysts compared to those derived from adult subjects, while OCT4 was significantly more abundant in adult derived blastocysts respect to prepubertal and parthenogenetic embryos (ANOVA;  $P < 0.05$ ). The relative abundance of Nanog mRNA in adult derived blastocysts was significantly higher compared to prepubertal (ANOVA;  $P < 0.05$ ) and similar to parthenogenetic ones.

Our data indicate that the up regulation of IFN-tau in prepubertal and parthenogenetic embryos could be related to the *down-regulation* of Oct4 and Nanog and may express an unbalanced developmental switch to the trophectoderm. Such different patterns of expression of in vitro produced blastocysts from prepubertal or adult oocytes may be related to the generally observed reduced in vitro and in vivo developmental competence.

**Dr Ana Catarina Belejo Mora Torres**

Batista, Mariana; Diniz, Patrícia; Mateus, Luísa; Lopes da Costa, Luís

PhD Student

Faculdade de Medicina Veterinária - UTL, Portugal

**Effects of co-culture with luteal cells and oil overlaying on *in vitro* bovine embryo development**

This experiment was designed to evaluate the interacting effects of the presence of luteal cells and of mineral oil overlaying in the *in vitro* development of bovine embryos in a semi-defined medium. Upon IVM and IVF, cleaved embryos over two cells (n=600) were IVC in SOFaa + 5% serum in a modified atmosphere (90%N<sub>2</sub>, 5%CO<sub>2</sub>, 5%O<sub>2</sub>), between days 2 to 7 (day 0 = fertilization). Embryos were randomly allocated to four groups (n=150): a) embryos in SOFaa overlayed with mineral oil (E+O); b) embryos in SOFaa (E); c) embryos co-cultured with luteal cells in SOFaa overlayed with mineral oil (E+CL+O); d) embryos co-cultured with luteal cells (E+CL). At day 7, embryos were classified for stage of development and quality and medium was recovered to measure progesterone (P<sub>4</sub>) concentrations. Embryo development above compact morula stage was significantly lower (p<0,05) in group E (16%) than in the other groups (E+O=29%, E+CL+O=34%, E+CL=35%). Also the percentage of freezable embryos (quality I e II) was significantly lower (p<0,05) in group E (62%) than in the other groups (E+O=84%, E+CL+O=96%, E+CL=89%). There was a tendency (p<0,1) for embryos in group E+CL to be less advanced in development (%blastocyst + expanded blastocyst: E+O=66%, E=54%, E+CL+O=61%, E+CL=36%). P<sub>4</sub> concentrations were significantly higher (p<0,0001) in groups without oil overlaying than in groups with oil overlaying: a 50-fold increase in P<sub>4</sub> concentrations was present in groups without oil overlaying compared to groups overlayed with oil. In conclusion, oil overlaying improved development of embryos cultured alone but had no effect on development of embryos co-cultured with luteal cells. This suggests that oil overlaying allows for the removal of putative toxic substances generated by embryo metabolism, as it is also admitted in the case of somatic cell co-culture. P<sub>4</sub> produced by luteal cells was retrieved by oil but this had no influence on cell and embryo development.

**Mr Pablo Bermejo Álvarez**

Dimitrios Rizos, Pat Lonergan and Alfonso Gutiérrez-Adán

PhD Student

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**mtDNA copy number during bovine preimplantation development**

Mitochondria are the most abundant organelles in the mammalian oocyte and early embryo. At these stages, mitochondrial dysfunction, such as reduced copy number, could lead to infertility as they play an important role in oxidative phosphorylation and they must be present in sufficient number to segregate into the blastomeres. During the preimplantation period each mitochondrion contains one copy of mtDNA, which facilitates quantification by qPCR. We aimed to analyze mtDNA copy number at different stages of preimplantation bovine development. We collected and stored individually 15 oocytes and in vitro produced embryos at each stage analyzed (immature oocyte, matured oocyte, 2-cell, 4-cell, 8-16-cell, morula, blastocyst, expanded blastocyst and hatched blastocyst). Each individual oocyte or embryo was digested with proteinase K and mtDNA was analyzed by qPCR. Two different standard curves were used in each qPCR to avoid interassay variation. To simplify the results, the  $2^{-\Delta CT}$  method was applied, by subtracting the highest mean CT value of all the groups from every other value and then log-transforming the data. No statistical differences (one-way ANOVA) were found among different developmental stages. However, a slight increase was observed after maturation ( $1.55 \pm 0.3$  vs  $1.24 \pm 0.1$ ), followed by a decrease at the 2- and 4-cell stage ( $1.15 \pm 0.1$  and  $1.07 \pm 0.1$ ). After mtDNA transcriptional activation mtDNA copy number increased ( $1.55 \pm 0.1$  at 8-16-cell stage), being maintained at the morula, blastocyst and expanded blastocyst stages ( $1.59 \pm 0.1$ ,  $1.44 \pm 0.1$  and  $1.59 \pm 0.1$  respectively) and increasing after hatching ( $1.89 \pm 0.2$ ). These results suggest that the amount of mtDNA remains relatively constant until to the blastocyst stage. However, the dynamic changes suggest that mtDNA copy number is controlled by synthesis-degradation processes to ensure normal development.

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**The expression of peroxisome proliferator activated receptors (PPARs) genes in porcine endometrium during periimplantation period**

Peroxisome proliferators activated receptors (PPARs) belong to the nuclear receptor superfamily which play an important role as a transcriptional factors. Three isoforms of the receptor have been described as PPAR $\alpha$ , PPAR $\gamma$ , PPAR $\delta$ . Recently, their role has been characterized in reproduction. The aim of the present study was to determine PPARs mRNA expression in the endometrium during periimplantation in pigs with surgically separated uterine horns.

Under general anesthesia one of the uterine horn of each prepubertal gilts (n=20) was cut transversely and endings were closed by suture. The uterus consisted of one intact uterine horn connected to the uterine corpus and the second horn was detached from the uterus corpus but connected with the contiguous ovary. After 10 days recovery, gilts were synchronized and superovulated by single intramuscular injection of PMSG followed by hCG. The gilts were divided into two groups: the first group was inseminated 24 h after hCG treatment and then at 12 h intervals. The second group was used as control with surgical procedure but not inseminated. Animals of both groups were slaughtered at the 14<sup>th</sup> day of pregnancy or 14<sup>th</sup> day the estrous cycle, respectively. The mRNA expression of PPARs in endometrium was analyzed by real-time RT-PCRs. During pregnancy mRNA expression of PPAR $\alpha$  and PPAR $\delta$  was significantly higher in the horn containing embryos compared to contralateral horn where embryos did not developed. In controls, the expression of these genes did not differ between both horns. The expression of PPAR $\gamma$ 1 gene during pregnancy did not differ between both horns however was significantly higher compared with both horns derived from controls. The results indicate the role of PPARs during early stages of gestation in pigs, however further experiments have to be performed to establish importance of the PPARs.

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**Dr Luisa Bogliolo**

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**In vitro or in vivo post-fertilization embryo culture affects the gene expression patterns of ovine embryos**

Although the oocyte origin can play a crucial role in determining the developmental competence, the post-fertilization embryo culture environment may affect the blastocyst yield and quality.

Aim of this study was to examine the relative transcript abundance of a panel of developmentally important genes in in vitro or in vivo cultured ovine embryos derived from prepubertal or adult animals. Cumulus oocyte complexes derived from ovaries of regularly slaughtered prepubertal and adult sheep were matured and fertilized in vitro in standard conditions. Thirty hours post-fertilization, both prepubertal and adult early cleaved embryo groups (two cell stage) were divided in two cultural systems: in vitro (SOF + aa + 0.4% BSA in 5% CO<sub>2</sub> and 5% O<sub>2</sub>) or in vivo (in ewe oviduct). Seven days post-fertilization, three groups of 8 blastocysts for each class were recovered and used for gene expression analysis by reverse transcription followed by Real Time PCR. The relative quantification was performed with the 2<sup>-ddCt</sup> method (Livak and Schmittgen, 2001 Methods 25, 402-8), after normalization against the  $\beta$ -actin expression levels.

Adult in vitro cultured embryos showed higher abundance for NaKATPase and Aquaporin 3 transcripts than in vivo counterparts (ANOVA; P<0.05), while no difference was detected for OCT4, E-cadherin, IGF2R and IGF2 mRNAs for embryos cultured in the two systems. Prepubertal in vitro cultured embryos showed higher expression of NaKATPase and IGF2R transcripts than in vivo cultured ones (ANOVA; P<0.05), and similar abundance for OCT4, Aquaporin 3, E-cadherin and IGF2 mRNAs.

Since embryos belonging to both classes were produced by in vitro fertilization of in vitro matured oocytes, the post-fertilization embryo culture environment itself seems to affect the gene expression pattern of important genes at the blastocyst stage. Furthermore, the observed expression patterns highlight a different response to culture environment for embryos deriving from adult or prepubertal oocytes.

**Dr Tiziana Brevini**

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**Mammalian parthenogenetic cell lines display abnormal chromosome complements and aberrant centriole number**

Mature oocytes can be activated in vitro leading to the generation of pluripotent cells that show the standard features associated with their biparental counterpart. However many aspects still need to be elucidated and, in particular little attention has been addressed so far to the incidence of aneuploidy in these cells. In mammals, with the possible exception of mouse, the lack of the paternal contribution to the parthenote centrosome is considered as the most likely cause of aneuploidy.

In this study we analysed the rate of aneuploidy and centriole distribution (as a marker of centrosome anomalies) in cell lines derived from pig and sheep parthenotes and in sheep parthenotes foetus that reached their maximum development in vivo (27 days).

Centriole number and distribution was assessed both by immunocytochemical analysis and with ultrastructural evaluation. Karyotyping was performed on mitotically active cells. Chromosomes spreads were evaluated and processed using the Leica CW4000 Karyo software.

The results obtained indicate that cell lines of parthenogenetic origin have, in all examined cases, an incidence of aneuploidy significantly higher than that of their respective controls with the majority of the cells displaying an hypo-haploid and hypo-diploid set up, possibly related to a loss of chromosomes during the mitotic process. A higher incidence of multiple centrioles was also detected, suggesting the hypothesis that aneuploidy may be related to the lack of paternal contribution that results in abnormal centrosome formation, incorrect control of the process of spindle rearrangement and consequent chromosomes malsegregation. Abnormal segregation and multicentriolar distribution was not limited to cell lines but was observed in parthenogenetic foetus, indicating that culture artifacts are unlikely to be the cause.

## Expression of adiponectin receptors during mouse preimplantation development

Cell receptors play a key role in communication between early embryo and maternal environment. Our research is focused on the expression of heptahelical membrane receptors using the model of mouse preimplantation embryo *in vivo* and *in vitro*. In the present study we examined the expression of receptors for adiponectin, a cytokine (adipokine) secreted from adipose tissue into the bloodstream. Recent data suggest that, besides the influence on maternal health, adiponectin can also play a role in the regulation of female reproductive system and early embryo development. To ascertain if adiponectin can directly interact with cells of preimplantation embryos, we investigated expression of adiponectin receptors in mouse preimplantation embryos *in vivo*. The expression of mRNAs for adiponectin receptors AdipoR1 and AdipoR2 was examined with RT-PCR in oocytes, 4 - cell embryos, 8~16 - cell embryos, morulas, and blastocysts. The transcripts were then quantified with real time RT-PCR using three different approaches for transformation of fluorescence data to the relative quantity – relative standard curve method, linear regression analysis of fluorescence data, and fitting fluorescence data with the sigmoid curve. All the three techniques showed similar results: mRNAs for both receptors are expressed in ovulated oocytes, then the quantity of both mRNAs drops, around morula stage it increases again and reaches maximum at the blastocyst stage. Expression of the receptors at protein level was examined by immunohistochemistry. The immunofluorescence signal for both receptors was found in all tested stages from oocyte to blastocysts, with higher intensity for AdipoR2 protein. Our results indicate that the two adiponectin receptors – AdipoR1 and AdipoR2 are expressed during mouse preimplantation development, which suggests a possibility of direct interaction of adiponectin with cells of these embryos.

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**Mrs Maria Clemente**

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**Transcriptional differences at the initiation of elongation in bovine conceptuses derived *in vivo* or *in vitro***

The majority of embryonic mortality in cattle occurs before maternal recognition of pregnancy at Day 16 post conception. *In vitro*-derived embryos exhibit a higher incidence of loss than their *in vivo*-derived counterparts. The aim of the present study was to compare transcriptome profiles in bovine conceptuses at the initiation of elongation derived either *in vitro* or *in vivo*. Day 7 bovine blastocysts were produced either *in vitro* by maturation, fertilization and culture or *in vivo* by superovulation, AI and non-surgical embryo recovery and were transferred in groups of 10 to synchronized heifers (10 recipients per group) ipsilateral to the CL and Day 13 conceptuses, recovered by flushing the uterus at slaughter were individually snap frozen in liquid nitrogen. Differential expression and Gene Ontology overrepresentation analyses were performed using BioConductor. In total, 288 genes were found to be differentially expressed (>1.5-fold;  $P < 0.05$ ), of which 133 were up-regulated and 155 down-regulated in *in vivo* compared to *in vitro* conceptuses. Analysis of the GO terms associated with differentially expressed genes showed genes involved in transport (23%), metabolic process (13%), transcription (13%), protein modification process (9%), multicellular organismal development (9%), cell differentiation (9%), signal transduction (4%), translation (4%), protein transport (4%), protein metabolic process (4%), biosynthetic process (4%) and cell proliferation (4%). Interestingly, transcripts such as Apolipoprotein A-II (*APOA2*), Lipoprotein lipase (*LPL*), and Fatty acid binding protein 7 (*FABP7*), known for their role in fatty acid uptake, transport, and metabolism, were up-regulated (>3 fold) in *in vivo* conceptuses. In addition 6 transcripts of the Solute Carrier (*SLC*) group of membrane transport proteins were found to be up-regulated (>2 fold) in *in vitro* conceptuses. In conclusion these findings indicate a qualitative and quantitative alteration of major biological functions and expression of individual genes in conceptuses derived *in vitro* versus *in vivo* which may be associated with the establishment of pregnancy in cattle.

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**Mr Thomas Connolly**

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### Calcium signalling during sperm-female tract interaction

Recent data suggests the female tract alters gene expression after exposure to sperm. We have used single-cell fluorescence imaging to observe calcium signalling in both human sperm and human reproductive tract cells upon initial contact and during sperm adhesion and release.

Explants and primary cell lines were prepared from donated human reproductive tract tissue removed during surgery. An immortalised oviductal cell line (*OE E6/E7*) has been used as an internal standard. Human sperm were selected by their ability to migrate through a viscous medium (~ 140 centipoise) and then resuspended in sEBSS media containing 0.3% FBS (charcoal stripped). Sperm were incubated for at least 3 hours at 37°C 6% CO<sub>2</sub> before use.

To investigate whether there was rapid cell signalling occurring in human female tract cells upon exposure to sperm, tract cells were labelled with Calcium Green-1, AM to monitor intracellular calcium levels. Sperm were labelled with Syto64, a red fluorescent nuclear dye, to allow tracking of sperm movement and contact with cells. Values are percentage change in fluorescence  $\pm$ S.E.M.

Initial data with tract cells show that explants demonstrated transient responses of  $9\pm 5\%$  (n=3) and an average peak duration of ~ 20s. Primary tract culture responses were  $15\pm 10\%$  (isthmus, n=3),  $7\pm 4\%$  (ampulla, n=3), both with similar peak durations of ~ 30s. *OE E6/E7* cells had larger responses of  $22\pm 10\%$  (n=6) and generated transients with an average peak duration of ~ 35s. Human sperm bound to and interacted with cells from all tract zones. Sperm swimming patterns appear to be modified when in the presence of reproductive tract cells.

Finally, we investigated whether calcium signals occurred within sperm whilst in contact with tract cells. Sperm were loaded with Calcium Green-1, AM. Preliminary evidence suggests calcium signals may relate to observed motility changes.

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**Characterization of novel olfactory receptors**

Sperm motility is regarded as crucial for natural conception, and also important in some instances of assisted conception, but to date there is still no accurate diagnosis of sperm motility and also no specific drug treatment available to improve sperm cell function.

The identification of odorant receptor (OR) expression in mammalian testicular tissue in the 90's set the grounds for a potential role of these chemoreceptors in significant sperm behaviours relevant for fertilization. More recently one of these receptors has been characterised and bourgeonal was shown to be a strong agonist for the receptor and act as a potent chemoattractant in behavioural bioassays with human spermatozoa.

Recent work within our group revealed that nitric oxide produced in the female reproductive tract can S-nitrosylate sperm. Additional proteomic approaches on this research identified two candidate ORs as targets for S-nitrosylation, the function of which is still unknown. One further candidate has also been identified in other distinct proteomic screenings of sperm and is therefore likely to be prevalent.

Our aim is to decipher the potential role of these ORs in sperm and explore eventual possibilities of sperm modulation. To this end we have assembled DNA constructs for candidate gene expression in a HEK293 cell system to allow high-throughput screening of potential odorant ligands via intracellular calcium ( $[Ca^{2+}]_i$ ) imaging of responses to agonists/antagonists and further functional characterisation. To date we have narrowed the active ligands to a mixture of 10 odorant compounds from an initial mixture of 100 compounds for two receptors.

**Dr Maria Dattena**

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**Preliminary study on irradiation effects in  
*in vitro* produced vitrified sheep embryos**

Cryopreserved biological material is exposed to natural background irradiation (estimated of 1 mGy per year) which might induce untoward effects. The purpose of this study was to evaluate the irradiation effects in *in vitro* produced vitrified embryos with doses simulating different interval of times.

Ovaries of Sarda sheep were collected from slaughterhouses. The oocytes were matured in TCM-199 with 4 mg/ml BSA for 24 h and fertilized with fresh ram semen. Zygotes were cultured in 20 µl droplets of synthetic oviduct fluid (SOF) with 8 mg/ml BSA until blastocyst stage (day 6-7). All 60 obtained embryos were vitrified and plunged in liquid nitrogen (- 196 °C), 52 were exposed at three different irradiation doses (<sup>137</sup>Cs γ-rays, 50mGy/sec) and 8, no irradiated, were used as control. The irradiation groups were divided according to the following doses: 18 embryos were irradiated with 0.3 Gy, 24 with 2.4 Gy, 10 with 19.2 Gy. They were all transferred in pairs into synchronized ewes. Pregnancy was confirmed by ultrasonography at day 40 and carried out until term.

Pregnancy rates were 55% (5/9), 42% (5/12), 0% (0/5), 75% (3/4) and lambing rates were 28% (5/18), 21% (5/24), 0% (0/10), 50% (4/8) for 0.3, 2.4, 19.2 Gy and control group respectively. Gy 0.3 group had low mortality and almost normal pregnancy and survival rate, while Gy 2.4 group had higher mortality when compared with control group. In addition, one out of 5 born lambs showed abnormal limbs and two died within two hours from lambing due to several organ malformations (emphysema, pneumonia, hydrapericardium, fill bladder, hepatitis). As expected, no embryo survived in 19.2 Gy group.

In conclusion, these results suggest that a low dose of irradiations do not produce significative effects, but a high dose might induce both embryo loss and abnormality followed by lamb death.

### **The effect of maternal intoxication on the development of mouse preimplantation embryos**

The aim of this study was to evaluate the possible effect of maternal poisoning by sub-lethal doses of Basta 15 on the development and the quality of mouse preimplantation embryos. Basta 15 is a liquid herbicide commonly used for weed control in agriculture. Its effect was tested using both *in vitro* and *in vivo* models. Stereomicroscopical evaluation of embryos, which were subjected to long-time culture (from early stages to the blastocysts stage) in media supplemented with herbicide, proved the ability of Basta 15 to negatively affect their developmental capacities. There were significant differences in the proportion of blastocysts, slowly developing embryos and degenerated embryos between controls and embryos cultured with Basta 15 at concentration 0.001% and higher. Furthermore, the concentration 0.001% significantly increased the incidence of apoptotic cells (evaluated by fluorescence viability staining combined with TUNEL assay) in short-time (24h) cultured blastocysts. Concentrations over 0.01 % caused developmental arrest (i.e., significant decrease in average cell number per blastocyst) and massive degeneration of blastomeres. During *in vivo* tests, adult female mice were synchronized, fertilized and fed by various doses of Basta 15 diluted in 200 µl of water every other day for one week. Embryos were collected 24h after the last application of herbicide. Stereomicroscopical evaluation of embryonic pools obtained from treated mothers showed that Basta 15 at dose 58 µl/kg (which refers to 1/50 of mouse LD50) and higher negatively affected their developmental capacities. The percentages of obtained blastocysts were significantly decreased and the percentages of slowly developing and degenerated embryos were significantly increased. Moreover, the presence of herbicide in maternal body had negative effect on blastocyst quality. Results of both tests show that the maternal intoxication with Basta 15 might strongly affect the developmental abilities of preimplantation embryos.

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**Epigenetic status of H19 in chorioallantoic tissues of sheep embryos produced by assisted reproduction technologies (ART)**

ART can lead to epigenetic alterations, including an incorrect imprinting status of specific genes. *H19* is a paternally imprinted gene, which epigenetic status can be deregulated by *in vitro* procedures. In this study the expression and methylation of *H19* has been analyzed (by real time quantitative RT-PCR and methylation specific PCR) in chorioallantois obtained from *in vitro* fertilized (IVF) and somatic cell nuclear transfer (SCNT) embryos, as well as from parthenogenotes (PA) (i.e. diploid embryos having only maternal genes), and from controls, obtained by natural mating. All analysis has been carried on chorioallantoic tissues obtained from alive (heart beating) fetuses on 18, 20, 22 days of gestation. PA samples have been divided for either alive and not alive (without heart beating) embryos, because sheep PA embryos are usually dying around this gestational stage. *H19* mRNA level was deregulated in all experimental groups: down-regulated in IVF and SCNT ( $P < 0.05$ ) and up-regulated in alive PA ( $P < 0.01$ ). *H19* mRNA was not expressed in not alive (without heart beating) PA in contrast with alive ones ( $P < 0.05$ ), in which expression was three fold over control. Surprisingly, chorioallantois from not alive PA still displayed normal mRNA expression of two other genes (VEGF and DNMT1). In spite of their exclusively maternal DNA origin, traces of methylated form of *H19* locus were noted in all alive PA samples. Furthermore, in 14 % of IVF chorioallantoic tissues an altered *H19* imprinting status, demonstrated by the lack of methylated allele, was detected. In conclusion, we have demonstrated that the epigenetic status of *H19* is affected by ART, and, its deregulation can be associated with an extended survival observed in some PA sheep.

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**Does interleukin 1  $\beta$  stimulate luteotrophic PGE<sub>2</sub> synthesis and secretion by porcine endometrium and myometrium during early pregnancy in pigs?**

Pig conceptuses secrete interleukin 1 $\beta$  (IL1 $\beta$ ) which has been proposed to serve as the initial stimulus for embryos elongation and their attachment to the uterine surface. We examined if IL1 $\beta$  regulates synthesis and secretion of luteotrophic PGE<sub>2</sub> in the pigs. Individual endometrial and myometrial slices harvested on days 10–11, 12–13 and 15–16 of pregnancy and the estrous cycle were placed in culture vials containing 2 ml of supplemented Medium 199, pre-incubated (18h, 37°C, 95% O<sub>2</sub> and 5% CO<sub>2</sub>) and next treated for 6 and 12 h with control medium and IL1 $\beta$  (1 or 10 ng/ml). After incubation, medium was collected for PGE<sub>2</sub> assay. In uterine slices, expression of the mRNA encoding mPGES-1 was investigated. Secretion of PGE<sub>2</sub> by uterine slices was affected by: dose IL1 $\beta$ , F = 55.4, P = 0.0000001), time of in vitro culture with IL1 $\beta$  (F = 67.62, P = 0.0000001) and days of reproductive status (F = 5.74, P = 0.004). The endometrial response to IL1 $\beta$  was the highest on days 12–13 in gravid pigs and on days 10–11 and 15–16 in cyclic pigs. In gravid pigs, myometrial response to IL1 $\beta$  was statistically higher on days 10–11 and 12–13 than on days 15–16. In non-gravid pigs, myometrial release of PGE<sub>2</sub> in response to IL1 $\beta$  was about ten-fold and five-fold higher on days 10–11 and 15–16 than on days 12–13, respectively. IL1 $\beta$  increased: 1. endometrial abundance of mRNA for mPGES-1 during studied days of the pregnancy and on days 15–16 of the estrous cycle (P<0.04 and P<0.002, respectively); 2. myometrial mRNA for mPGES-1 on days 10–13 of pregnancy and on days 10–11 and 15–16 of the estrous cycle. IL1 $\beta$  acts as the stimulator of uterine luteotrophic activity during maternal recognition of pregnancy and luteal phase of the estrous cycle.

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### **The effect of embryos presence on myometrial and endometrial secretion of PGE<sub>2</sub> in response to IL1 $\beta$ and TNF $\alpha$ in pigs**

Periimplantation period in the pig is critical for successful establishment of pregnancy. The influence of embryos on IL1 $\beta$  and TNF $\alpha$  regulated myometrial and endometrial secretion of luteotrophic prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) on days 12-14 of pregnancy in pigs was studied. We used unilaterally pregnant pigs created by surgical procedure in which one uterine horn remained intact and the second horn was cut transversely. Thus, a part of the second horn was detached from the uterine body. Animals were divided into two groups: inseminated (slaughter on days 12-14 of pregnancy, n=6) and uninseminated cyclic gilts, used as controls (slaughter on days 12-14 of the estrous cycle, n=5). Embryos developed only in the patent part of the uterus and not in the occluded horn. Myometrial and endometrial slices were cultured in vitro in the presence of IL1 $\beta$  (10 ng/ml) or TNF $\alpha$  (10 ng/ml). The basal myometrial PGE<sub>2</sub> secretion was higher in the intact gravid uterine horn than in non-gravid uterine horn during pregnancy and did not differ between parts of the uterus during the estrous cycle. The basal endometrial secretion of PGE<sub>2</sub> during pregnancy and the estrous cycle was higher in the isolated non-gravid uterine horn than in the intact uterine horn. In the myometrium, IL1 $\beta$  stimulated secretion of PGE<sub>2</sub> from both parts of uterus only in pregnant pigs, while endometrial secretion of PGE<sub>2</sub> was increased in intact gravid uterine horn of pregnant pigs and in intact uterine horn of cyclic pigs. TNF $\alpha$  increased myometrial and endometrial secretion of PGE<sub>2</sub> both in intact gravid and in intact non-gravid uterine horn of pregnant pigs. In cyclic pigs this effect was observed only in the endometrium of intact uterine horn.

Embryonic signals regulate myometrial secretion of luteotrophic PGE<sub>2</sub> in pigs. IL1 $\beta$  and TNF $\alpha$ -induced secretion of PGE<sub>2</sub> was increased in myometrium during pregnancy independent on the presence of embryos in particular parts of uterus. The cytokines promote luteotrophic actions of PGE<sub>2</sub> in porcine uterus.

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**Miss Rebecca Frettsome**

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**Modulation of human sperm by the cumulus oophorus**

Current sperm preparation techniques such as density gradient and swim-up methods do not accurately reflect the physiological challenges sperm face through the female reproductive tract. During their journey ejaculated sperm must pass through a number of barriers, finally to reach the oolema and fertilise the oocyte, sperm must penetrate a layer of granulosa-derived cells called the cumulus oophorus and the zona pellucida (ZP). The presence of the cumulus cell layer appears to be a critical factor in fertilisation, previous studies have shown that the cumulus cells release a complex range of factors such as progesterone, nitric oxide (NO) and chemokines that have an effect on sperm motility and their ability to undergo the acrosome reaction.

We have studied the effects of sperm interaction with native human cumulus/granulosa cells from women undergoing assisted reproduction treatment and an immortalised human granulosa cell line (COV434) in a system which mimics the physiological environment of the female reproductive tract. Our results suggest that rather than just being a passive barrier through which the sperm must pass to reach the oocyte, the cumulus plays an active role in sperm selection and activity modulation. A better understanding of the interaction of sperm with the cumulus-oocyte complex will aid the optimisation of assisted reproduction techniques.

**Dr Antonio Gonzalez-Bulnes**

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**Incidence of embryo mortality and intrauterine growth retardation in a swine model with obesity and leptin resistance (Iberian pig)**

The Iberian pig is an ancient breed, autochthonous of the Mediterranean area, with a high potential for fat accumulation, causing obesity, due a leptin resistance syndrome related to leptin receptor gene polymorphisms. Iberian pig is affected by a lower prolificacy when compared to lean breeds. Current study aimed to determine possible differences in rates of ovulation and embryo implantation and later incidence of embryo mortality and intrauterine growth retardation (IUGR) in sows of Iberian breed (n= 23) and highly-prolific lean commercial crosses (Large White × Landrace, n= 17) at two critical periods of swine pregnancy: Days 21 (just after achievement of trophoblast attachment) and 35 (just after completion of the transition from late embryo to early fetal stage). There were identified two different reproductive performances in the Iberian breed; a 69.5% of the females had lower ovulation rates than LWxL ( $22.5 \pm 1.6$  vs  $13.2 \pm 2.3$ ,  $P < 0.05$ ), but a 30.5% had similar number of ovulations ( $18.2 \pm 3.9$ ). However, these Iberian sows having high ovulatory rates showed a high incidence of regression of corpora lutea and embryo losses between Days 21 and 35, which was not found in Iberian females with low ovulation rates; so, finally, the number of viable embryos was similar in both Iberian groups ( $8.2 \pm 1.0$  and  $8.4 \pm 1.0$ ) and lower that in highly-prolific LWxL ( $14.8 \pm 1.8$ ,  $P < 0.05$ ). At Day 35, a total of 167 conceptuses (73 LWxL and 94 IB) were evaluated for IUGR. The LWxL conceptuses were longer and wider than Iberian ( $69.5 \pm 0.1$  and  $64.4 \pm 0.1$  vs  $49.9 \pm 0.1$  and  $41.9 \pm 0.1$  mm,  $P < 0.0001$ ); however, Iberian conceptuses were heavier than LWxL ( $107.4 \pm 6.6$  vs  $68.6 \pm 2.4$  g); which can be related to the macroscopic perception of a lower quantity of fluids and a higher development of the placental tissues in comparison to the embryo itself.

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## Influence of culture conditions on the embryonic expression of growth factor receptors

The *in vitro* embryo culture environment is still suboptimal to the complex *in vivo* environment, and both short- and long-term side effects have been described due to *in vitro* culture conditions. The continuous interaction between the maternal reproductive tract and the embryo allowing exchange of nutrients and signaling molecules is completely lost in an *in vitro* culture system.

As little is known about the response of preimplantation embryos on growth factors, the influence of culture conditions on the embryonic expression of growth factor receptors (GFRs) was determined.

First, the mRNA expression of five GFRs (*IGF1R*, *IGF2R*, *PDGFRA*, *INSR*, *CSF2RA*) was measured on *in vitro* embryos at different time-points of development. *IGF1R*, *IGF2R* and *INSR* showed a relatively high maternal mRNA expression that was declining towards the 9-16 cell stage and reactivated around the morula stage. *PDGFRA* and *CSF2RA* had an overall very low mRNA expression level.

Next, the mRNA expression of these GFRs was compared between three groups of 5-8 cell embryos (*in vivo* embryos, *in vitro* embryos matured and cultured with serum and *in vitro* embryos matured and cultured without serum). At this developmental stage, the embryos are depending on maternal transcripts and *in vivo* embryos are residing in the oviduct. For *IGF1R* and *IGF2R* no differences were found between the three groups. A significantly higher *INSR* mRNA expression was found in both *in vitro* embryo groups compared to the *in vivo* group. *PDGFR* had a twofold higher expression in the *in vitro* + serum group and *CSF2RA* was lower expressed in *in vivo* embryos.

In a future experiment, the expression of these GFRs will be determined at the blastocysts stage, after activation of the embryonic genome and when the embryos are residing in the uterus.

**Dr Ghylène Goudet**

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**Are Oviductin, Osteopontin and Atrial Natriuretic Peptide A expressed in equine oviduct and involved in equine fertilization?**

In mammals, the mechanism of fertilization remains puzzling. The secretory cells of the oviduct synthesize proteins that interact with gametes and promote *in vitro* fertilization (IVF) in human, bovine and porcine species. In the equine species, despite numerous attempts, the IVF rates remain low. We showed previously that oviductal epithelial cell (OEC) co-culture improves equine IVF rates (Mugnier et al., 1<sup>st</sup> Cost Action, 9-11 October 2008, Volos, Greece). The aim of our study was to identify the proteins secreted by the oviduct that could be responsible for this positive effect on IVF.

First, we searched for the presence of the genes encoding oviductin, osteopontin and Atrial Natriuretic Peptide A (ANP A) in the equine genome on the basis of phylogenetic analysis. We showed the presence of the genes encoding osteopontin and ANP A, but for oviductin the gene has been lost.

Then, we searched for the presence of osteopontin and ANP A proteins in the equine oviduct fluid using gel electrophoresis and immunoblotting. We showed the presence of both proteins in equine oviducts throughout the oestrus cycle.

Finally, we analyzed the influence of osteopontin and ANP A on equine IVF. Equine oocytes were *in vitro* matured for 30 hours. Equine sperm was incubated with calcium ionophore. Gametes were co-cultured without or with osteopontine (from 0.01 to 100µg/ml) or ANP A (from 0.1 to 50nmol/l) during 24 hours, and analyzed for signs of fertilization. No significant effect of osteopontin or ANP A on equine IVF could be highlighted.

In conclusion, OEC co-culture improves equine IVF rates, but up to now, we did not clearly identify the proteins involved. The oviductal secretions may be of particular importance for fertilization in this species, and the horse may be an interesting model to clarify their role in the mechanism of sperm-egg interaction.

**Prof Roberto Gualtieri**

Valentina Mollo, Vincenza Barbato, Riccardo Talevi  
Associate Professor  
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**Ex vivo sperm-oviduct adhesion and release in cattle**

Different models have been developed to study the interaction of gametes and embryos with the maternal reproductive tract in vitro. Although collected data have been considered representative of the in vivo physiological processes, the physiology of in vitro models has often been questioned. In vitro studies on sperm-oviduct interaction in cattle demonstrated that oviductal monolayers bind sperm and prolong their viability. Bound sperm are released by sulfated glycoconjugates and disulfide reductants that have been suggested to represent the physiological sperm releasing signals. However, culture of monolayers is accompanied by signs of de-differentiation and the significance of results obtained with monolayers has been questioned. In the present study we examined sperm-oviduct adhesion and release in the nearest in vivo condition. Oviducts were slit open longitudinally and fixed with metal pins on the bottom of Petri dishes. In experiment 1, single oviducts were divided into three consecutive segments and inseminated with hoechst-labeled sperm in TALP in 10cm Petri dishes to study the regionality of sperm binding.

In experiment 2, isthmic and ampullary segments were inseminated and then treated with heparin 100 $\mu$ g/ml, penicillamine 100 $\mu$ M or TALP alone. Data from Exp 1 showed that sperm binding density is higher in the ampulla than in the isthmus. In the ampulla sperm bound both the longitudinal folds and the pockets between folds, whereas in the isthmus a higher sperm binding to the pockets between folds was observed. Data from exp 2 showed that heparin and penicillamine induced the release of sperm bound to both ampullary and isthmic segments.

In conclusion, 1) the whole oviduct mucosal surface is able to bind sperm and the ampullary region has a higher sperm affinity; 2) previously identified sperm releasing signals are effective also in this nearest in vivo condition.

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**Sperm-oviduct adhesion and release in the bovine species under culture conditions that highly promotes oviduct differentiation**

Different *in vitro* models have been developed to understand the interaction of gametes and embryos with the maternal reproductive tract. However, depending on the differentiation state, cell-cell interactions *in vitro* might be not representative of the *in vivo* condition. New, more physiological approaches of *in vitro* culture might improve the significance of studies on gamete and embryo interaction with the maternal reproductive tract at the cellular, transcriptomic, proteomic and secretomic level. Previous studies on sperm-oviduct interaction in cattle were carried out with oviductal monolayers (OM). Although OM show signs of de-differentiation such as the decrease of cell height, and the regression of ciliation and secretory activity they retain a number of features of the oviduct *in vivo*. Herein, OM were cultured in a semi-defined culture medium, in an immersed or a liquid-air interface condition, on microporous polystyrene or polycarbonate inserts coated with human placental collagen for 15-30 days after cell confluence. Scanning electron microscopy showed that OM cultured in a liquid-air interface condition were composed of cubic-columnar ciliated and secretory cells. Bound sperm viability was maintained significantly better at 48hr post insemination than in conventionally cultured parallel OM. Molecular signals that induce the release of sperm bound to conventionally cultured OM were equally effective on sperm bound to differentiated OM. OM whose basal surface was treated with estradiol bound significantly more sperm than untreated OM.

In conclusion, 1) modified culture promotes a highly differentiated condition as appreciated by cell morphology, ability to maintain the motility of bound sperm and response to estradiol; 2) the action of sperm releasing signals on sperm bound to OM cultured with the conventional or the modified method is equally effective; 3) culture of OM under conditions that highly promote differentiation may provide an *in vitro* model that mimicks more closely the biological processes modulated by the oviduct *in vivo*.

**Dr Marta Hernandez**

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**Effects of the heat shock 70 kDa protein 8 (HSPA8) on boar sperm motility**

The mammalian oviductal environment plays an essential role in ensuring the availability of a highly fertile sperm population suitable for fertilization. However the complex mechanisms involved in prolonging sperm survival in the female reproductive tract remain unclear. Oviductal apical plasma membrane proteins (sAPM) are able to enhance *in vitro* sperm survival and suppress boar sperm motility in a dose-dependent manner. The survival enhancing property has been partially attributed to the heat shock protein (HSPA8). However, relationships between the protective effects of HSPA8 and sperm motility are poorly understood. The present study investigates the modulatory effects of HSPA8 (SPP-751; Stressgen) on the bicarbonate-induced motility activation response of boar spermatozoa. Washed boar spermatozoa were pre-incubated in the presence or absence of HSPA8 (5, 0.5, 0.05 and 0  $\mu\text{g protein mL}^{-1}$ ) for 10 min before adding 15mM bicarbonate/CO<sub>2</sub> (or 15 mM NaCl as a control). Sperm motility was observed before bicarbonate/CO<sub>2</sub> or NaCl addition, at 2 min after bicarbonate/CO<sub>2</sub> addition and at 5-min intervals up to 17-min. A final observation of NaCl-treated control spermatozoa was performed at 22-min. Data from 42,251 individual spermatozoa were obtained from nine replicates (boars); individual sperm motion parameters were analyzed and subjected to PATN analysis which classified them into four groups. Group 2 was identified as a fast-linear subpopulation, whereas the others were slow and non-linear. The relative frequencies of fast-linear spermatozoa were compared between treatments. This showed that HSPA8 inhibited the rate of bicarbonate-induced motility stimulation; 5  $\mu\text{g/mL}$  showed the most pronounced effect and 0.5 and 0.05  $\mu\text{g/mL}$  were roughly equivalent. In addition, the maximum degree of motility stimulation was inhibited in a dose dependent manner. These findings suggest that HSPA8 within the oviduct actively regulates subpopulation-specific sperm motility activation, an effect that might be linked to the establishment and maintenance of the oviductal sperm reservoir.

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**Creating a favorable reproductive tract environment  
for successful fertilization**

Reproduction involves interaction between male and female derived molecules and cells at many levels. In addition to direct sperm-egg interactions, sperm also interact with structures of the upper and lower reproductive tract while eggs may interact directly with the oviduct as well as other structures in the reproductive tract. Equally critical interactions are likely to occur between gametes, molecules in the seminal fluid, and molecules produced by reproductive tract epithelial and glandular tissues. These interactions act in concert to effect successful fertilization. The fruit fly *Drosophila melanogaster* has become a major model organism for studying these interactions, which not only ensure successful fertilization but also mediate behavioral and physiological effects that reflect conflicting evolutionary pressures on the two sexes.

We have shown that females up-regulate transcription of numerous genes just after mating that may influence subsequent oocyte maturation and sperm maintenance. To gain a better understanding of how mating-responsive genes contribute to the function of the female reproductive tract, we analyzed genes predicted to have a role in maintaining the upper and lower reproductive tract microenvironment. Using the UAS-GAL4 system, we examined how female fertility is affected when selected genes were silenced in the reproductive tract. These results, in conjunction with morphological and localization studies, may allow us for the first time to begin to address how the microenvironment is maintained and/or regulated in the female reproductive tract.

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**Protein expression of lysophosphatidic acid receptor 3 gene in porcine endometrium during the periimplantation period and estrous cycle**

In pigs, accurate embryo spacing determines the litter size, pre- and postnatal development however, factors responsible for this mechanism remain unknown. Our previous studies, based on gene expression suggested, that like in rodents, lysophosphatidic acid receptor 3 (LPA3) may influence early stages of pregnancy in pigs. Determination of gene expression in the endometrium of unilaterally pregnant pigs showed higher mRNA level in the gravid uterine horn compared to the non-gravid horn. Moreover, increased LPA3 transcript level was observed during pregnancy on days: 6-7, 8-9, 11-12 and 13-14 in comparison with the corresponding days of the estrous cycle. Thus, the aim of the presented study was to determine the level of LPA3 protein in the same periods. Similarly, higher protein expression was observed in the endometrium of the gravid uterine horn compared to the non-gravid horn and both horns of control animals (not inseminated gilts). Protein level did not differ significantly between the investigated days of the estrous cycle, whereas during pregnancy increased on day 11, sustained high to day 17, and then markedly decreased. Moreover, only in the period between day 11 and 17 of pregnancy –protein level was significantly higher comparing to the corresponding days of the estrous cycle. During the mentioned period of swine pregnancy initiation of implantation occurs. The obtained data confirm LPA3 gene expression and indicate the importance of LPA3 receptor on protein level as well. Thus, we conclude that LPA3 may play important role during embryo spacing and the implantation process which determines considerably further embryo development.

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**Can corpus luteum be a target for lysophosphatidic acid action during early pregnancy in cattle**

We have recently shown that LPA synthesized in the bovine endometrium stimulates prostaglandin (PG)E<sub>2</sub> and progesterone (P4) secretion during luteal phase of estrous cycle and early pregnancy *in vivo*. LPA concentration and mRNA transcript expression for LPA receptor type 1 (LPA1) are higher in the bovine endometrium during early pregnancy than during estrous cycle. Therefore, the objective of the present study was to examine if the bovine corpus luteum (CL) may be a target for the action of LPA produced in the endometrium during early pregnancy. Before slaughter, cows were divided into two groups: pregnant and cyclic. The animals chosen for the pregnant group underwent artificial insemination (AI). The tissues for this experiment were assigned for the following days of the estrous cycle and pregnancy: days 2-4 of the cycle (n=5), days 8-10 of the cycle (n=7) and pregnancy (n=12), days 17-19 of the cycle (n=4) and pregnancy (n=5). Localization and expression patterns for LPA receptors (LPA1, LPA2, LPA3, LPA4) in the bovine CL were evaluated. Immunohistochemical studies localized proteins for all types of LPA receptors in the membranes of luteal cells and in some blood vessels during all examined phases of estrous cycle and early pregnancy. Quantification of mRNA for all examined LPA receptors in the CL did not differ during the estrous cycle (P>0.05). However, on days 8-10 and 17-19 of early pregnancy LPA1, LPA2, LPA3 and LPA4 transcript abundance was significantly higher than on the respective days of the estrous cycle (P<0.05). In conclusion, the present study demonstrated dynamic expression of the receptors for LPA in the bovine CL during the estrous cycle and early pregnancy. The overall data suggest that the bovine CL may be a target for LPA action during early pregnancy, thus might contribute to the development and maintenance of the CL in cattle.

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**Ovum Pick Up and ICSI allow to overcome infertility of mares affected by a variety of pathological conditions of the reproductive tract**

In the last few years, assisted reproductive technologies have been developed also in the horse following their successful application in several farm animal species. In particular, the technique of Ovum Pick Up (OPU) combined with intracytoplasmic sperm injection (ICSI), in vitro embryo culture (IVC) and embryo transfer can potentially allow to obtain offspring from subfertile or infertile mares that cannot reproduce by natural mating, artificial insemination or conventional embryo transfer (ET). In order to verify this possibility we have conducted a study in which we have performed 163 OPU-ICSI-IVP sessions, of which 80 from healthy mares and 83 from donor mares with reproductive problems, and we have evaluated the outcome in terms of embryo production and pregnancy rate comparing Reproductive problems included cervix laceration, uterus aplasia, endometritis, embryo resorption, no embryo production with conventional ET, abnormal oocytes, history of no pregnancy. Were also included in this group mares with more than 20 years. The study was performed both during and outside the breeding season.

We found no statistically significant difference between healthy donors and donors affected by reproductive pathologies in the rate of oocyte maturation (584/881: 66.28% vs. 628/967: 64.94%), cleavage (362/584: 61.98% vs. 413/628: 65.76% of injected oocytes) embryo development (69/362: 19.06% vs. 64/413: 15.49% of cleaved oocytes) and pregnancy rate (24/46: 52.17% vs. 25/36: 69.44%) following single transfer of frozen-thawed embryos.

Therefore, we conclude that, similarly to what has been already demonstrated in several other species, OPU-ICSI-IVP is an efficient and reliable technique that allows to overcome the infertility of mares affected by a variety of reproductive pathologies that compromise oocyte maturation/fertilisation and/or embryo survival and pregnancy establishment in the donor reproductive tract.

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**Evaluation of the ovarian reserve in prepubertal ewes  
is predictive of in vitro embryo yield**

Current study aimed to investigate if differences observed in post-natal ovarian follicular development in sheep could be related to differences in oocyte quality and in the ovarian reserve, as evaluated by ultrasound antral follicle count (AFC), and by the exogenous FSH ovarian reserve test (EFORT), performed by a single-shot administration of 60 mg FSH. AFC in pre-pubertal ewes aged 30-40 days evidenced significant differences in follicle numbers ( $p < 0.0001$ ) and three different groups could be identified: high ( $40.7 \pm 7.8$  follicles), intermediate ( $20.5 \pm 3.1$ ) and low ( $14 \pm 1.3$ ). Significant differences were also observed after the EFORT test, when a higher number of follicles was recorded in the high group ( $56.7 \pm 17.8$ ) as compared to the intermediate ( $31.6 \pm 12.7$ ) and low ones ( $26.3 \pm 13.4$ ;  $p < 0.001$ ). At the same time, ovaries from pre-pubertal ewes slaughtered at 30-40 days of age were collected to evaluate oocyte *in vitro* developmental competence. Ovaries were classified in the same experimental groups according to follicle number. A higher number of oocytes/ovaries were selected for IVM from the high group ( $40.8 \pm 18.2$ ) compared to the intermediate ( $10.4 \pm 4.5$ ) and low ones ( $1.1 \pm 0.6$ ;  $p < 0.0001$ ) and were fertilized ( $31.8 \pm 14.9$  vs  $8.2 \pm 3.5$  and  $0.9 \pm 0.4$ , respectively;  $p < 0.05$ ). No differences were recorded in cleavage rates. A faster developmental rate was observed in the high compared to the intermediate and low groups, as evidenced by higher percentage of blastocysts developed by Day 7 post-fertilization (8.6% vs 4.5 and 1.1% respectively;  $p < 0.05$ ). Finally, total blastocysts output was significantly higher in high and intermediate groups (20.6 and 17.2%, respectively) compared to the low one (6.6%;  $p < 0.05$ ). This study evidenced that ovarian status deeply influences oocyte quality as soon as a few weeks after birth. Further studies are needed to investigate whether differences recorded in ovarian reserve could eventually predict reproductive performance after puberty.

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**Effects of P<sub>4</sub>, OT, PGE<sub>2</sub> and Growth Factors on equine luteal cells production of progesterone and prostaglandin E<sub>2</sub>**

The main function of corpus luteum (CL) is production of progesterone (P<sub>4</sub>) which is essential for the establishment and successful of pregnancy. Mechanisms controlling secretory function of the CL may involve factors that are produced, both within and outside the CL and ovary. It is also well documented that prostaglandin (PG)E<sub>2</sub>, oxytocin (OT) and growth factors i.e.: vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF) may serve as local luteotropic factors stimulating P<sub>4</sub> secretion by the CL of several species. However, there is limited number of study documented the local mechanisms regulating the secretory function of equine CL. The objective of this study was to determine whether P<sub>4</sub>, OT, VEGF and IGF influence on P<sub>4</sub> and PGE<sub>2</sub> production by the equine cultured luteal cells *in vitro*. Mare's CL were collected at the local slaughterhouse and classified as the mid-luteal phase CL (n=4). Isolation of the luteal tissue was done by the enzyme solution. The cells were seeded at a density of 2 x 10<sup>5</sup> viable cells/ml and cultured in 24-well culture plates. After 8 hours incubation the cells attached to the plate and the medium was replaced by fresh incubating medium. Luteal cells were exposed for 24h to medium only – control or to: P<sub>4</sub> (10<sup>-6</sup>M), OT (10<sup>-7</sup>M), PGE<sub>2</sub> (10<sup>-7</sup>M), IGF (50 ng/mL) and VEGF (50ng/mL). Cell conditioned media were assayed for P<sub>4</sub> and PGE<sub>2</sub> by EIA. P<sub>4</sub> production increased after stimulation with OT (p<0.05) and PGE<sub>2</sub> (p<0.01). The secretion of PGE<sub>2</sub> increased after stimulation with P<sub>4</sub>, OT, IGF and VEGF (p<0.05).

These data suggest that OT, P<sub>4</sub>, PGE<sub>2</sub> and growth factors (VEGF, IGF) may play important role as a auto-paracrine factors regulating secretory CL function and may interact as a lutotropic factors at time when the early embryo development occur and the maintains of CL is necessary.

### **In vitro survival and viability of bovine morulae after vitrification in cryoloop**

Vitrification is a relatively rapid and inexpensive cryopreservation procedure, and it has been shown to be useful for in vitro-produced embryos that have lower cryosurvival. The present study aimed to vitrify bovine morulae using cryoloop procedure and to assess subsequent in vitro development. Oocytes were matured, fertilized and cultured in vitro in a serum free system. At day 6, morulae were divided in two groups: embryos vitrified in cryoloop; non vitrified embryos. For vitrification, embryos were first exposed to 5 M ethylene glycol (EG) in Hepes-Synthetic Oviductal fluid (HSOF) for 3 min at room temperature, then moved to 7 M EG in HSOF for 20 sec; then morulae were allocated onto the nylon loop (Hampton Research, Laguna Niguel, CA, USA), and immediately plunged in liquid nitrogen. Embryos were warmed by immersing the loop in 2 ml of dilution solution (Galactose 0.5M in HSOF), washed in HSOF and cultured in SOFaaBSA (16 mg/ml) plus 5% FBS at 38.5°C in 5%O<sub>2</sub>, 5%CO<sub>2</sub>, 90%N<sub>2</sub>. After 48 h hatching rate was recorded and the expanded blastocysts were stained with Hoechst 33342 and PI. Chi Square test and ANOVA were used for statistical analysis. Vitrification of bovine morulae in cryoloop did not affect survival rate but blastocyst rate was lower for vitrified than fresh control morulae (% re-expansion: 100.0%; % blastocysts: 51.2% vitrified embryos vs 73.3% control). No significant differences were found in the hatching ability (23.3% vs 31.9%), and in the number of live, dead and total cells (Live cell: 100.6±35.8 vs 104±10.2; dead cell: 12.4±5.1 vs 8.5±2.9; total cell: 114.7±35.0 vs 112.2±11.5). In conclusion, cryoloop vitrification of in vitro produced bovine morulae is a valuable method for cryopreservation, with a satisfying survival rate, embryo quality and developmental ability.

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**Identification of different heat shock proteins in the porcine pre-ovulator oviductal fluid**

Under physiological conditions, oviductal fluid is the milieu where fertilization takes place. In the *in vitro* situation, culture media try to mimic the oviductal fluid function. However, culture media lack of proteins present in the oviduct that could be important for the fertilization success. Recent studies have reported the relevance oviductal epithelial cell proteins in sperm capacitation and fertilization. Some of these proteins are heat shock proteins (HSP) and it was described that the sperm viability and *in vitro* fertilization efficiency are influenced by these proteins. Additionally, a recent study reported that HSP is present in the sperm plasma membrane and is involved in the zona pellucida binding. In the present study, the presence of HSP in the oviductal fluid was investigated. Oviductal fluid collected from sows near the ovulation time were centrifuged, separated by 1D gel electrophoresis and processed for proteomic analysis, which was carried out on an HPLC/MS system using an ESI interface. The proteomic analysis indicates the presence of the following HSP: HSP90AA1, HSP90AB1, HSP70 protein 1A, HSP70 protein 1B, HSP70 protein 5, HSP70 protein 8, HSP70 protein 12B and the HSP 105 kDa.

This study demonstrates the presence of different HSP proteins in the oviductal secretions suggesting that these proteins are probably secreted by the epithelial oviductal cells; however, the hypothesis that these proteins are coming from death cells present in the oviductal lumen is not discarded. Further analytical studies will be performed to validate the results obtained. Due to the relevant role played by some these HSP previously reported in the gamete interaction, fertilization and sperm viability further *in vitro* fertilization studies with the presence of each of the individual identified proteins or combination of these HSP in the culture medium could help to elucidate the contribution of these proteins to the fertilization process.

**Investigation of developmental competence of bovine oocytes from antral follicles of different sizes and selected with brilliant cresyl blue**

Follicular oocytes recovered from slaughterhouse material for in vitro embryo production are extremely heterogenous in terms of quality and developmental competence. The percentage of oocytes reaching the blastocyst stage in a completely in-vitro system still varies. Mammalian oocytes selection on the basis of visual assesment of morphology of the cumulus-oocyte complex (COC), have led to limited improvement in the identification of oocyte quality. Recent studies have shown that the staining of COC with brilliant cresyl blue (BCB), for glucose-6 phosphate dehydrogenase activity may be used to select developmentally competence of oocytes before maturation [1].

This study was conducted to evaluate development competence of oocytes, recovered from different follicles size, by staining with BCB.

Oocytes were recovered from slaughterhouse ovaries by aspiration from large (10-15 mm) and medium (5-9 mm) and by slicing the surface of ovaries from small (2-4 mm) follicles. All collected COC, except for markedly atretic oocytes were used. Oocytes were placed into three groups based on follicular diameter: (1) 2-4 mm; (2) 5-9 mm; (3) 10-15 mm. The oocytes were exposed to 26  $\mu$ M BCB in PBS containing 0,4% BSA for 90 min. and classified according to their cytoplasm coloration: BCB+(colored cytoplasm, low G6PDH) and BCB- (colorless cytoplasm, increased G6PDH). Oocytes were processed through standard procedures for in vitro maturation, fertilization and culture. Embryos were cultured to day 8.

The percentage of oocytes stained by BCB+ was 36.7 % (407/1109). The highest proportion of BCB+ oocytes – 67.6% was determined for oocytes recovered from large follicles, meanwhile, the lowest proportion - 27.9% was determined for oocytes recovered from small follicles. We observed high fertilization and cleavage rates in all groups of oocytes (53.8- 76%). The BCB+ oocytes in all treatment groups yielded a significantly higher proportion of blastocysts (24-39.1%) than did BCB-oocytes (1.9-9%).

*Alm H. et al. 2005. Theriogenology 63, 2194-2205*

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**Effects of leptin on maternal ovarian function and oocyte developmental competence in a mouse model of obesity/type 2 diabetes**

Current study used a leptin deficient model of obesity/type 2 diabetes (B6.V-Lepob/ob/OlaHsd; ob/ob mice) for elucidating reproductive effects of leptin, and its role at hypothalamus-hypophysis or at ovarian level. The ovulatory response and the fertilization success after controlled ovarian stimulation and ovulation induction were determined in 6 ob/ob adult females pretreated with a leptin-replacement therapy (serial doses of 10µg/g of body weight for 8 days) and compared with the results obtained in 6 ob/ob dams pretreated with a gonadotrophin-replacement therapy (0.5 IU of pure FSH and LH for 5 days; avoiding the hypogonadotrophic state but not mimicking possible local effects of leptin) and two control non-pretreated groups (six ob/ob females and 6 wild-type non leptin-deficient C57BL/6J mice). The number of corpora lutea was significantly lower ( $8.0 \pm 2.9$ ;  $P < 0.05$ ) in control ob/ob mice than in wild-type dams ( $21.3 \pm 3.4$ ). Treatment with gonadotrophin-replacement therapy did not increase significantly the ovulation rate in ob/ob mice ( $10.4 \pm 1.2$ ) but the administration of leptin-replacement treatment allowed to obtain a number of corpora lutea similar than obtained in wild-type females ( $17.8 \pm 3.7$ ). Afterwards, the controlled ovarian stimulation failed to produce fertilized zygotes in ob/ob mice without replacement therapy and in the group treated with gonadotrophin-replacement therapy. On the other hand, the gonadotrophin-replacement therapy succeeded in producing fertilized zygotes ( $6.4 \pm 3.4$ ), although in a lower number that found in the wild-type control ( $14.5 \pm 1.3$ ;  $P < 0.05$ ). Thus, current results strongly support, beside effects at hypothalamus-hypophysis axis, the existence of direct local effects of leptin on the ovary by direct *in vivo* observations.

**Distribution pattern of cortical granulae in oocytes  
collected from peri-pubertal gilts in  
relation to BCB competence and donor puberty**

In vitro production of porcine embryos gains a special interest due to its potential biomedical applications. The efficiency of the IVF procedure is however not satisfactory and the main obstacles are high rate of polyspermy and reduced embryo quality. The developmental potential (quality) of oocytes is a complex trait and can be characterized by parameters related to ooplasm and nucleus. Cortical granules (CG) play a basic role in establishing the polyspermy block. CG are absent from the cortex of non-mature porcine oocytes and their migration occurs during maturation. Therefore a proper CG distribution during IVM significantly affects their functions (exocytosis).

The aim of this study is to investigate an association between sexual maturity of the oocyte donor (peri-pubertal gilts) and CG distribution pattern in *in vitro* matured oocytes. Cumulus-oocyte complexes (COC) are collected from ovaries of commercially slaughtered young gilts at age of 6-7 months. The ovaries are divided into 2 groups: pre-pubertal (NCL - lack of *corpus luteus*) and pubertal (CL - presence of *corpus luteus*). COCs of a proper morphology are further selected by the BCB test and subjected to IVM. Six groups of oocytes will be analyzed: NCL BCB+, NCL BCB-, CL BCB+, CL BCB-, NCL control (non-BCB treated) and CL control oocytes. CG distribution pattern will be investigated in non-mature and mature oocytes (*Romar et al Anim Reprod Sci 2005:85*). Up to now images from 40 oocytes after IVM (3 sections per oocyte) were analyzed under a confocal microscope. The preliminary data that concerns mature oocytes of the non-BCB treated group shows no significant differences in CG distribution between CL and NCL gilts. All the investigated images presented a similar distribution pattern with a dense rim of CG localized under the oolemma.

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**Growth hormone affects transcript level of genes related to embryo quality in blastocysts derived from early cleaving bovine zygotes**

IVM conditions significantly affect developmental competence (quality) of oocytes, whereas growth hormone (GH) stimulates in vitro oocyte maturation and embryonic development in cattle. It has been shown that bovine zygotes that cleave early are characterized by a better quality than their later cleaving counterparts. Therefore, the aim of this study was to determine whether GH supplemented to IVM medium affects transcript level of a set of 5 genes described as potential markers of embryo quality in bovine blastocyst derived from early cleaving zygotes. COCs were matured in TCM-199 supplemented with Na-pyruvate, antibiotics, fafBSA and FSH. Experimental medium was enriched with GH. Mature oocytes were fertilized according to Parrish et al. (1998) whereas embryos were cultured in sequential G1.3/G2.3 media. All embryos that cleaved by 30hpi (early cleavers, EC) were selected and cultured separately. The remaining embryos cleaved by 48hpi (non-early cleavers, NEC) were incubated in separated drops. At 180 hpi, hatched blastocysts were pooled and subjected to RNA isolation and RT-QPCR (LightCycler Roche) as described by Warzych et al. (2007). H2a has been used as reference gene. Transcript level of the control H2a gene was constant in all samples. EC experimental embryos were characterized by the highest relative transcript abundance (RA) of the Hsp 70.1 gene, the lowest Bax to Bcl-2 transcript ratio, and lower RA of the stress (p66shc) and apoptotic related (Bax, survivin) genes when compared to the control. Experimental and control NEC blastocysts did not differ with regard to RA of all analyzed genes. In conclusion, GH present during IVM significantly improved the quality of EC blastocysts by decreasing RA of pro-apoptotic (Bax, survivin) and oxygen stress related (p66shc) genes as well as by increasing RA of Hsp 70.1 gene.

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**Performance of luteal cells in an *in vitro* culture system designed for bovine *in vitro* embryo production**

In order to develop an *in vitro* culture model to study the interactions between early bovine embryos obtained by IVM-IVF and luteal cells, we evaluated the progesterone (P4) production (as a measure of cell viability and function) of luteal cells in optimal conditions to IVC such as hypoxic (5% O<sub>2</sub>+5% CO<sub>2</sub>), hyperthermia (39°C) and in minimal semi-defined media (SOF+aa).

The cells, at an initial seeding of 1×10<sup>5</sup> cells/well, were cultured in DMEM-F12 in a 20% O<sub>2</sub> and 5% CO<sub>2</sub> atmosphere at 39°C, in 48 well plates. After 72 hours (D0) the medium was aspirated and wells were randomly allocated to receive TCM 199, DMEM-F12 or SOF with 5% FCS and culture proceeded in 5% O<sub>2</sub> plus 5% CO<sub>2</sub> atmosphere at 39°C for 5 more days. Half the wells were refreshed at day 3 of culture and samples were obtained on days 0, 3 and 5 for P4 measurement by a RIA method. Results of 5 sessions and several replicates per session were analysed by the Kruskal-Wallis test.

Early CL (days 1-5 of the oestrus cycle) cells produced significantly more P4 (P<0.0001) than Mid CL (days 6-12) cells in the 3 media. P4 production was not significantly affected (P>0.05) by media. Also, P4 concentrations on day 5 were not significantly different for cells with the medium refreshed on day 3 or for cells with undisturbed culture until day 5.

In conclusion, luteal cells can be cultured under hypoxic and hyperthermic conditions, in a minimal medium, keeping the P4 production capability, which is useful for a co-culture *in vitro* model to study the early embryo-luteal cells interactions.

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**Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows**

Infertility in dairy cattle is a multifactorial problem which may be linked to follicle development and the quality of the ovulated oocyte, sperm transport and fertilization and/or the reproductive tract environment. The aim of this study was to compare the ability of the reproductive tract of postpartum dairy cows and nulliparous heifers to support the development of early embryos to the blastocyst stage. Two- to four-cell bovine embryos (n=1800) were produced by in vitro maturation and fertilization of oocytes derived from the ovaries of slaughtered cattle. The oestrous cycles of nulliparous Holstein heifers (n=10) and postpartum Holstein cows (n=8, approximately 60 days post partum) were synchronized using an 8 day CIDR insert coupled with prostaglandin injection. Only animals seen in standing oestrus (=Day 0) were used. On Day 2, 100 2- to 4-cell embryos were endoscopically transferred to the oviduct ipsilateral to the corpus luteum. Five days later, on Day 7, the oviduct and uterus were flushed non-surgically and the number of embryos developing to the blastocyst stage was recorded. A representative number of blastocysts from heifers and cows were stained to assess cell number. Progesterone concentrations were lower ( $P<0.05$ ) in cows than heifers on Days 5, 6 and 7. Significantly more embryos were recovered from heifers than cows ( $79.0\pm 7.0$  vs  $57.2\pm 11.4\%$ ,  $P<0.05$ ). Of the structures recovered,  $33.9\pm 3.6\%$  had developed to the blastocyst stage in the heifer oviduct compared to  $18.3\pm 7.9\%$  in the postpartum cow oviduct ( $P<0.05$ ). There was no evidence of a difference in blastocyst quality as evidenced by total cell number in the blastocysts ( $71.2\pm 5.7$  vs  $67.0\pm 5.3$ , respectively). In conclusion, the reproductive tract of the post partum lactating dairy cow may be less capable of supporting development of the embryo that that of the non-lactating heifer and this may contribute to the lower conception rates observed in such animals.

**Dr Daniela Sanna**

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**Quantitative analysis of Oct4 in sheep embryonic stem-like cells**

*Oct4* is a POU domain transcription factor that is specifically expressed in all pluripotent cells during early embryonic development and it also plays an important role in embryonic stem cells acting as a master regulator of the genes that keep the cells in an undifferentiated state. This study was conducted to quantitatively detect *Oct4* gene expression in sheep embryonic stem (ES)-like cells at the undifferentiated stage and in the course of differentiation (ECD) in order to establish and strengthen its pluripotent role also in this species.

Oocytes were obtained from ovaries of slaughtered adult ewes, matured in vitro in TCM-199 with 4mg/ml fatty acid-free BSA for 24h and then fertilized with fresh ram semen (day 0= fertilization day). Zygotes were cultured for 6-7 days in synthetic oviduct fluid (SOF) supplemented with 8 mg/ml fatty acid-free BSA at 39°C under 5% CO<sub>2</sub>, 7% O<sub>2</sub> and 88% N<sub>2</sub>. On the third and the fifth day of culture 8 mg/ml fatty acid-free BSA were added to the culture. Twelve blastocysts were used to produce 6 ES-like colonies (50%) according to Dattena et al. (2006). Three colonies were isolated from the feeder layer after 5-7 days to obtain ES like-cells and 3 were isolated after 8-10 days to obtain ECD cells. Granulosa cells (GR) were used as negative control. Poly(A)mRNA was extracted from ES like-cells, ECD and GR using 300 cells for sample and relative mRNA abundance was analyzed by Quantitative RT-PCR and normalized to the housekeeping gene GADPH.

The relative mRNA abundance of ovine *Oct-4* was markedly higher in ES-like cells compared with ECD showing a significant difference between groups ( $P < 0.05$ ), while it was undetectable in GR.

This study demonstrates that undifferentiated ES-like cells and their corresponding differentiated cells differ in their level of *Oct4* expression thus strengthening the role of *Oct4* as pluripotent cell marker.

### **Effect of LH and steroids on leptin gene expression in the porcine luteal cells during early pregnancy**

Leptin, the product of the *ob* gene, is involved in the regulation of reproductive functions in different species, including pigs. It can act through hypothalamic–pituitary–gonadal axis, after binding to its receptors. At ovary level, the leptin might directly influence ovulation process, corpus luteum formation and ovarian steroidogenesis. Recently, it was shown leptin gene and protein expressions in porcine corpus luteum during early pregnancy (days 14-16 and 30-32, the beginning and the end of implantation) and a key role for leptin during this period has been suggested. Leptin is involved in synthesis of hormones and molecules which regulate trophoblastic invasion, embryo growth and development. Study with *ob/ob* mice has revealed that exogenous leptin supplementation to the mother was required at least for 6,5 days post coitum to retain pregnancy. Moreover, recent evidence indicates that the leptin transcript level can change during the oestrous cycle and pregnancy. Thus, it may imply that expression of this gene depends on concentration of circulating steroids or LH.

The aim of these studies were to examine the effects of LH, E<sub>2</sub> and P<sub>4</sub> on leptin gene expression in the porcine luteal cells during early pregnancy (days 14-16). Isolated luteal cells after preliminary culture (48 h) were treated for 24 h with LH, E<sub>2</sub> and P<sub>4</sub>. Total RNA was isolated from cells and transcribed into cDNA. The expression of leptin mRNA was analysed by Real-Time PCR methods.

It was shown that LH (10 ng/ml) and E<sub>2</sub> (0.02 and 0.2 ng/ml) significantly stimulated leptin gene expression in the porcine luteal cells on 14-16 days of gestation. It has not been observed significant effect of P<sub>4</sub> on leptin mRNA expression in the cells. These data indicate that LH and steroids are involved in the regulation leptin mRNA expression in the porcine luteal cells during early pregnancy.

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**Intra-uterine transfer of equine cleaved embryos**

Although transferrable equine *in vitro* blastocysts can be produced, equine *in vitro* culture systems are still suboptimal. Temporary transfer of day 2-3 cleaved embryos to equine or ovine oviducts has been used to increase blastocyst rates. However, this requires surgery and is therefore less practical. Another way to culture embryos *in vivo* is premature intra-uterine transfer of early cleavage stage embryos, which is successfully being performed in human medicine. The aim of this study was to evaluate the outcome of intra-uterine transfer of cleaved embryos in horses.

Oocytes from slaughtered mares were matured *in vitro*, fertilized by ICSI and cultured for 2-3 days in DMEM-F12 with 10% FCS or 5% FCS and 5% SR in 5% O<sub>2</sub>, 5% CO<sub>2</sub> and 90% N<sub>2</sub>. A total of 105 cleaved embryos were transferred to the uterus of 12 synchronized mares (average of 8.75 embryos / mare). Half of the mares were examined by ultrasound 14 days after ICSI and half were flushed at 7 days.

No pregnancies were diagnosed in the first group. The group of flushed mares yielded no blastocysts. In 3 flushings an embryo was recovered, but in all cases it was degenerated.

According to this study the equine uterus is not a suitable environment for cleaved equine embryos. The developmental competence of early embryos which are prematurely transferred to the uterus appears to be unique for primates. In domestic animals however the oviduct seems to play a vital role. Another explanation could be an intrinsic lack of developmental competence of the transferred *in vitro* embryos, although *in vitro* equine blastocysts and pregnancy after blastocyst transfer have been produced with the same protocol.

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**Spermatozoa DNA integrity after thawing  
strongly influences subsequent in vitro embryo output**

Several molecular and cellular markers have been proposed as a tool to evaluate sperm fertility in vitro in raw or processed semen samples, but with highly variable results. The aim of the present study was to investigate which parameters among a battery of analyses could predict frozen/thawed spermatozoa in vitro fertilization ability and hence blastocyst output. Ejaculates were obtained by artificial vagina from 3 adult bucks aged 2 years (A, B and C). In order to assess the predictive value of viability, ATP intracellular concentration and of DNA integrity, as evaluated by the comet assay, on subsequent embryo output after an in vitro fertility test, a logistic regression analysis was used. Freezing significantly reduced semen viability in all the samples analyzed ( $p < 0.0001$ ), but semen collected from A maintained a higher viability compared with B ( $p < 0.05$ ), while C did not differ from the others. While no difference was found among the three individuals in percentage tail DNA, olive tail moment was lower in B than in A and C ( $p < 0.01$ ). In addition, comet length was significantly higher in C than in A and B ( $p < 0.01$ ). No differences were observed in ATP concentration before and after thawing among the three individuals. Results of IVF test showed that spermatozoa collected from A and B lead to higher cleavage rates ( $0 < 0.01$ ) and blastocysts output ( $p < 0.05$ ) compared with C. Logistic regression analysis model explained a deviance of 72.4% ( $p < 0.0001$ ), directly related with semen viability before ( $p < 0.01$ ) and after thawing ( $p < 0.01$ ), and with two of the three comet parameters considered, i.e tail DNA percentage ( $p < 0.0001$ ) and comet length ( $p < 0.0001$ ). DNA integrity alone had a high predictive value on IVF outcome with frozen/thawed semen (deviance explained: 57.2%). The model proposed here represent one of the many possible ways to explain differences found in embryo output following IVF with different semen donors.

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**Preliminary studies on isolation, culture and cryo-conservation of the epithelial and stromal cells from the equine uterus - technical report**

Horse is a typical seasonal reproduction animal of long-day with the season in Poland and other east-north European country between March and September. It is impossible to study the endometrial functions during the off-season. Thus, if equine endometrial cells could be isolated under optimum conditions and then passaged and stored, it would be possible to study the physiology of the endometrium *in vitro* at any time. Therefore, we decided to establish the methodology for isolation, culture and cryo-conservation of endometrial cells (epithelial and stromal cells).

Uteri were obtained at a local slaughterhouse. Only the horn which was ipsilateral to the corpus luteum was used for the study. Epithelial cells were isolated by incubation twice at 37,5°C for 60 min with an enzyme solution (dispase I, DNase I in HBSS with 0.1% BSA). The cell suspension was pooled and washed 3 times by centrifugation and resuspended in culture medium (DMEM/Ham's F-12; supplemented with 10% calf serum). After removing the epithelial cells the endometrium was dissected from the miometrial layer and digested in the enzyme solution (collagenase, DNase I in HBSS with 0.1% BSA). After 40 minutes stirring, the cells suspension was collected, washed 3 times by centrifugation and resuspended in culture medium. The cells were seeded at a density of  $2 \times 10^5$  viable cells/ml. After reaching the 100 % confluence, the cells were passaged using trypsin. One part of the cells was used for cryo-conservation, the rest of cells was cultured. The properties of the stored and passaged endometrial cells were evaluated by their production of prostaglandin  $E_2$  and  $F_{2\alpha}$  in response to oxytocin and tumor necrosis factor- $\alpha$ . The homogeneity of the cells and contamination of the stromal and epithelial cell cultures were evaluated by immunofluorescent staining for specific markers of epithelial cells (cytokeratin) and stromal cells (vimentin).

**Prof Riccardo Talevi**

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**Three dimensional alginate-collagen matrix enhances the in vitro growth of isolated bovine follicles**

Recently, many efforts have been done to help young patients to preserve their fertility through the storage of their own germ cells before chemo-and/or radiotherapy partially or completely destroy their follicular reserve. Several cryopreservation protocols have been developed for whole ovary or ovarian cortical strip cryopreservation. Unfortunately, these techniques are associated with the risk to cryopreserve also malignant cells. This risk can be avoided only if the follicles are isolated from the ovarian stroma. On the other hand, the extracellular matrix plays a key role for the development and the fully competence of follicles in vivo. The development of technologies that support the growth and maturation in vitro of oocytes from isolated primordial follicles is attractive for both cancer fertility preservation and human assisted reproduction. Herein we investigated the effectiveness of three dimensional matrix alginate and alginate + collagen to support the in vitro growth of encapsulated isolated primary and secondary bovine follicles. To isolate follicles, cortical ovarian tissue was dissected in small pieces of 0.5mm x 0.5mm x 1mm, digested with collagenase type 1A, 1mg/mL, and DNase, 0.2 mg/mL, at 37°C for 45 min. Single follicles were collected under the stereomicroscope and encapsulated in alginate 2% or alginate 2% + collagen IV 0,3 mg/mL. The encapsulated follicles were cultured in Medium 199 + 20% FCS in 5% CO<sub>2</sub> atmosphere at 38 °C for ten days and the follicles growth was measured daily with computer assisted image analyzer. Results indicate that, at the end of the culture period, the average growth and vitality of follicles were 57.11% and 44% for 2% alginate and 70.32% and 71,4% for alginate 2% + collagen IV. In conclusion three dimensional matrix alginate + collagen better supports the in vitro growth of isolated bovine follicles.

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**Altered expression of DNA-methyltransferase I in extraembryonic tissues of sheep embryos produced by various assisted reproduction technologies (ART)**

Methylation of cytosine of CpG dinucleotides is an epigenetic regulator of gene expression which is essential for mammalian embryo and placenta development. Appropriate DNA methylation is required also for the correct regulation of genomic imprinting, a marking mechanism by which genes are repressed on one of the parental alleles. The DNA-methyltransferases have a key role in the establishment of methylation pattern and are required for two types of activity: *de novo* and maintenance of methylation. DNMT1 is considered the major maintenance of methyltransferase as it is the only DNMTs known to exhibit a strong affinity for targeting hemimethylated DNA. Inappropriate genomic methylation and the ensuing epigenetic alteration is a likely cause of developmental abnormalities induced by assisted reproductive technologies (ART). Therefore, in this study we have analyzed the expression of DNMT1 in sheep placentae obtained by various ART procedures. Extraembryonic tissues were collected during early pregnancy (day 20 and 22) from embryos produced by natural breeding (control), parthenogenesis (PA), *in vitro* fertilization (IVF) and Somatic Cell Nuclear Transfer (SCNT). The expression of DNA-methyltransferase 1 mRNA and protein levels were detected by Real Time PCR and Western Blot respectively. Extraembryonic tissues from IVF and SCNT embryos have shown a reduced DNMT1 mRNA expression ( $p < 0.05$ ), confirmed at the protein level, whereas in extraembryonic tissues from PA embryos an increase in mRNA expression level was detected. These results demonstrated an altered expression of DNMT1 in sheep early placentas collected from early ART pregnancies, suggesting that defective maintenance of methylation marks leads to developmental defects in ART.

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**Aberrant prostaglandin metabolism in the uterus of early pregnant cattle following somatic cell nuclear transfer**

Prostaglandins (PG) are important mediators of female reproductive events including fertilization and implantation. An unbalanced production and action of PG may cause reproductive disorders. Bovine somatic cell nuclear transfer (SCNT) pregnancies are frequently characterised by placental failure initiated at the onset of pregnancy prior to implantation and thus provide an interesting *in vivo* model for studying early embryo-maternal communication. We quantitatively analysed PG present in uteri of SCNT pregnancies (n=8) in comparison to normal IVF pregnancies (n=10). Additionally, endometrium and trophoblast tissue were examined regarding specific enzymes and receptors involved in PG generation and function. SCNT and IVF embryos were cultured under identical conditions to the blastocyst stage (day 7) until being transferred to synchronized recipients, which were slaughtered at day 18 of pregnancy. Uterine flushings were analysed for PG using LC-MS/MS, and endometrial and trophoblast RNA was extracted for qRT-PCR analysis. Uterine flushings from pregnancies obtained with SCNT embryos had significantly lower levels of PGI<sub>2</sub> (4423±1286 vs. 9709±907 pg/mL, *P*=0.006, measured as 6-keto PGF<sub>1α</sub>) and PGE<sub>2</sub> (978±232 vs. 1656±212 pg/mL, *P*=0.03) than pregnancies obtained with IVF embryos. PGF<sub>2α</sub> was also slightly lower in SCNT pregnancies, while the 15-keto metabolites of PGF<sub>2α</sub> and PGE<sub>2</sub> were significantly elevated (270±60 vs. 102±23 pg/mL, *P*=0.03, and 117±20 vs. 26±4 pg/mL, *P*<0.001, respectively). Day 18 trophoblasts normally synthesize high amounts of PG with marginal PG catabolism, whereas the endometrium has a higher PG metabolic turnover than the trophoblast. Interestingly, transcripts of PG synthases I and E were both lower in the endometrium of SCNT as compared with IVF pregnancies (1.7-fold, *P*<0.001 and 1.4-fold, *P*=0.05, respectively), while the mRNA of 15-hydroxyprostaglandin dehydrogenase forming 15-ketoprostaglandin metabolites was neither changed in trophoblast nor endometrial tissue. Protein verification will further disentangle the causes of unbalanced PG levels in the uterine lumen. An aberrant reciprocal embryonic and maternal contribution with possible adverse fertility consequences seems feasible.

### **Intrauterine expression of LIF and its receptors in the cycling and early pregnant mare**

Leukaemia Inhibitory Factor (LIF) plays a critical role in blastocyst development and implantation, as clearly demonstrated by the failure of wild-type mouse embryos to implant in the uterus of LIF-knockout female mice unless the latter receive LIF supplementation. Expression of LIF and its receptors (LIF-R and gp130) in the early pregnant horse have not been described. However, the horse may be an interesting animal in which to study such 'implantation factors' because various aspects of implantation occur both unusually late and in a temporally distinct fashion in this species.

The aim of this study was to examine LIF, LIF-R and gp130 gene expression in the endometrium of cycling and early pregnant mares, and in the early conceptus. Endometrial biopsies were recovered from 4 mares at each of late oestrus, days 7 and 14 of dioestrus (cycling mares), and days 7, 14 and 21 of gestation. Conceptuses were recovered by uterine lavage (day 7) or using a video-endoscopically guided net (days 14 and 21). For day 14 and 21 conceptuses, only the bilaminar trophoblast was used in gene expression studies. Expression of mRNA for LIF, LIF-R and gp130 was analysed by rtPCR, with relative expression calculated with respect to the 3 most stable housekeeping genes using GeNorm analysis.

A dramatic increase in LIF mRNA expression ( $p < 0.01$ ) was observed in both the endometrium and the trophoblast on day 21 of pregnancy. Expression of LIF-R and gp 130 increased significantly in the trophoblast on day 21 ( $p < 0.01$ ), but did not vary in the endometrium at the various stages examined. We propose that LIF plays a role in the adhesion between trophoblast and endometrium which, in the mare, cannot begin until the blastocyst capsule is dissolved somewhere between days 18 and 22 of gestation.

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**Expression of 15-hydroxyprostaglandin dehydrogenase in the porcine endometrium and trophoblast during early pregnancy**

Prostaglandin (PGs) are primary regulators of reproductive function. Biosynthesis and catabolism of PG influence concentration and availability of these biologically active hormones. The enzymes involved in PG synthesis have been intensively investigated in the porcine endometrium, corpus luteum and conceptus in the pig. However, reports referring to potential role of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the main enzyme of PG catabolism, are limited in the porcine endometrium and conceptus. The aim of the study was to evaluate 15-PGDH expression in the endometrium and in conceptus and to determine the concentration of 13,14-dihydro-15-keto PGF<sub>2α</sub> (PGFM) in endometrial homogenates and in uterine lumen during the estrous cycle and early pregnancy in the pig. Endometrial samples and uterine flushings were collected from gilts on days 9, 11, 12 and 14-16 of the estrous cycle (n=29) and pregnancy (n=22). Moreover, conceptuses and trophoblasts (n=20) were obtained from gilts on days 12-16 (n=6), 18-20 (n=7) and 22-25 (n=7) of pregnancy. Protein 15-PGDH was expressed in the endometrium on days 9-16 of the estrous cycle and pregnancy. On day 11 of pregnancy expression of 15-PGDH protein was above 2-fold higher in endometrium from mesometrial site when compared to antimesometrial site (p<0.05). Increase of 15-PGDH protein expression occurred in the conceptuses on days 18-20 and 22-25 of pregnancy vs. days 12-16 of pregnancy (p<0.01). Higher concentration of PGFM in the endometrium homogenates and uterine flushings was observed on days 14-16 of pregnancy when compared with the corresponding stage of the estrous cycle (p<0.01). Expression pattern of 15-PGDH protein in the conceptus as well as changes of PGFM concentration in the endometrium and uterine lumen after day 14 of pregnancy suggest contribution of 15-PGDH in modulation of PG catabolism during implantation in the pig.

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**Production of androgenetic sheep embryos**

Monoparental embryos are a precious tool to investigate parental contribution during early development. While the production of parthenogenetic embryos is easily performed in farm animals, deriving androgenetic ones is more complicated. We previously demonstrate that it is possible to obtain reasonable numbers of sheep androgenetic embryos only through pronuclear transfer between in vitro fertilized (IVF) enucleated oocytes (Matsukawa et al., 2007). We next clarified that the poor development of androgenotes produced by IVF of enucleated oocytes was attributable to the activation - and the ensuing block of polyspermy – induced during manipulation in a Ca<sup>++</sup> containing medium. In this work we optimized the protocol for the production of androgenotes by IVF of enucleated oocytes and characterized sheep androgenotes. At 22 h of maturation, oocytes were enucleated in PBS (Ca<sup>2+</sup>, Mg<sup>2+</sup> free) with BSA, Cytochalasin B and Hoechst 33342 and subsequently in vitro fertilized. Androgenetic embryos were also produced according the procedures described by Matsukawa et al (2007). Normally fertilized embryos were produced as control. After IVF, some presumptive zygotes were centrifuged at 13000Xg to identify the pronuclei (10-15 hours post IVF), while the others were transferred in SOF medium and cultured till blastocyst stage. Pronuclear numbers evaluated showed that 47.42% of zygotes were diploid. Blastocyst rate estimated at day 8 showed a similar developmental capacity between androgenetic embryos produced only by IVF of anucleated oocytes and those produced trough pronuclear transfer (18.8% vs 18.6% respectively). To further characterize the androgenetic blastocysts, chromosome number was analysed by Giemsa Stain. This analysis demonstrated a similar chromosome composition between IVF and androgenetic blastocysts. To conclude, this work demonstrates the possibility to produce diploid androgenetic sheep embryos using a simple protocol based on IVF of previously enucleated oocytes.

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**In vitro trophoblast differentiation from pig epiblast stem cells**

We have recently established the conditions for the derivation and culture of pig epiblast stem cells (pEpiSC) from in vivo embryos. pEpiSC depend on FGF and activin/nodal signalling for pluripotency and self renewal. These cells express *Oct-4*, *Nanog*, *Sox-2* and *Nodal*, but don't express *Rex1* and they are alkaline phosphatase negative, similar to features reported for mouse epiblast stem cells. pEpiSC can spontaneously differentiate into three germ layers when cultured without, and they can be efficiently directed towards neurectoderm using standard differentiation protocols. We have also established that pEpiSC respond to BMP4 stimulation by reactivating the germ cell markers *Dazl* and *Vasa*, indicating that these cells can commit to the germ cell program in vitro. BMP4 is also critical modulating differentiation of hESC into trophoblast. Here we show that pEpiSC can differentiate to trophoblast as we established by analysing the activation of *Eomes*, *msx2*, *H19*, *progesterone receptor* and *aromatase* after 7 days induction with BMP4 in serum free conditions. Current efforts are focussed in deriving trophoblast stem cell lines.

These results show for the first time that *bonafide* pig epiblasts can be used as a source of pluripotent cells, and that the signalling pathway maintaining pluripotency and self renewal is conserved between humans and pigs. We anticipate that these cells will have a variety of applications, among others, as in vitro models of trophoblast differentiation in ungulates.

**Dr Carmen Alminana**

Cristina Cuello, Jonatan Sanchez-Psorio, Inmaculada Parrilla, Maria Antonia Gil, Xiomara Lucas, Jose Luis Vazquez, Juan Maria Vazquez, Jordi Roca, Emilio Arsenio Martinez, Alireza Fazeli  
Postdoctoral Research Associate  
University of Sheffield, United Kingdom

**Pig embryo at blastocyst stage elicit a local response in sow endometrium**

Deciphering the complex cross-talk between a mother and her embryo is important. This basic reproductive biology knowledge has practical implications for human medicine and livestock production. General endocrine signalling pathways and local factors are both involved in mediating maternal-embryo interactions. But, the local factors are less defined. Here, we hypothesized that the presence of embryo at the blastocyst stage in the female reproductive tract induces local response(s) in the endometrium. A novel experimental model design together with a high-throughput microarray analysis technique was used to investigate the validity of this hypothesis in porcine species. 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 blastocyst stage 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 the endometrial tissue in the presence and absence of embryo using the Affymetrix Genechip Porcine Genome array. Bioinformatic analyses showed significant differences in gene expression between endometrium in presence and absence of embryos. Surprisingly, most differences in gene expression were concerned with genes being expressed in endometrium in the absence of embryos (horn with unfertilized oocytes). Further experiments are currently in progress to verify these data with other technologies such as real time PCR. In conclusion these data provide a clear evidence for existence of local responses in endometrium towards embryo at blastocyst stage. This study was supported by grants from Seneca foundation and MICINN.

**Prof Pilar Coy**

Irene Mondejar, Pilar Coy

**On behalf of Mr Luis Grullon**

PhD Student

University of Murcia, Spain

**Plasminogen/Plasmin system is involved in the regulation of polyspermy in the pig and cow**

Plasmin, the protease derived from the activation of plasminogen, has been related to the rebuilding or degradation of the oocyte zona pellucida during fertilization or early development (Roldan-Olarte *et al.* 2005). Plasminogen was detected in the oocyte plasma membrane and zona pellucida and we have found it in the oviductal fluid. Plasminogen activators (cell tissue-type, t-PA and urokinase-type, u-PA) have been detected in the oviduct, spermatozoon acrosome and oocyte cortical granules of different species. PA inhibitors are also present in the porcine oviduct (Kouba *et al.* 2000).

Using *in vitro* fertilization (IVF), sperm-zona pellucida binding and enzymatic assays, different experiments were designed to know more about the role of the plasminogen/plasmin system on the pig and cow sperm-oocyte interaction. The results showed that plasminogen concentrations similar to those in blood serum, added into the IVF medium, reduced significantly the sperm penetration and increased monospermy. This reduction was dose and time-dependent, and it was higher when plasminogen was added 30 minutes after IVF (70% of reduction in cow and 51% of reduction in pig) than when it was added 30 minutes before sperm-oocyte contact (52% of reduction in cow and 33% of reduction in pig). Production of plasmin was detected in the IVF medium containing plasminogen, oocytes and spermatozoa in both species, indicating that PAs were released from the gametes. Finally, the spermatozoa bound to ZP, evaluated after 15 min and 3 h of gametes coincubation did not decrease in the control medium ( $91.19 \pm 2.29$  and  $88.32 \pm 2.10$ , respectively, in the pig;  $3.97 \pm 0.18$  and  $3.29 \pm 0.22$ , respectively, in the cow), but they decreased significantly ( $P < 0.001$ ) in the presence of plasmin ( $26.46 \pm 1.08$  in pig and  $1.89 \pm 0.75$  in cow after 3 hours of coincubation). These results suggest that proteolytic action of plasmin produces the detaching of some spermatozoa attached to zona pellucida. This could contribute to the regulation of polyspermy.

**Mr Mahbubur Rahman**Maria Mazzilli<sup>2</sup>, Georgia Pennarossa<sup>1</sup>, Tiziana A.L. Brevini<sup>1</sup>, Alfonso Zecconi<sup>2</sup>, Fulvio Gandolfi<sup>1</sup>

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### Chronic mastitis is associated with a reduced development of ovarian follicles

Mastitis is associated with disruption of reproductive processes in dairy cattle but the mechanisms linking this disease with poor reproductive function are unknown <sup>1</sup>. We investigated the effect of chronic mastitis on the ovarian reserve and follicular dynamic. Ovaries and milk samples were collected at the time of slaughter. Quarter milk samples were analyzed following NMC procedures<sup>2</sup>. Based on the presence of major pathogens and somatic cell count results, animals were divided in two groups: healthy (n = 8) and affected by chronic mastitis (n = 9). Primordial, primary and secondary follicles were counted on similar surface area for each animal (mean  $\pm$  SD= 5,65  $\pm$  0.25 cm<sup>2</sup>).

The numbers of primordial, primary and secondary follicles were divided in 2 classes by the statistical software, based on respective frequency distribution, analysed with Fisher's exact test and the association between health status and follicles number was estimated by odds ratios.

Results are expressed as odds ratios  $\pm$  confidence limits for the association between follicle number classes in the ovarian area analysed for each animal and udder health status.

Type of follicles (threshold)	Odds ratio confidence limit	95% inferior confidence limit	95% Superior
Primordial (<100)	2.08	0.298	14.549
Primary (<12)	0.50	0.070	3.550
Secondary (<2)	10.50*	1.115	98.914

\* = significantly different (P< 0.05)

Data provide preliminary evidence that chronic mastitis is associated with a significant reduction of the ability of primary follicles to develop into secondary follicles and is consistent with the hypothesis that this pathology disrupts reproductive functions through products of the microorganisms colonizing the mammary gland or by cytokines and other bioactive molecules produced in response to infection.

1 Hansen, P.J., et al. Am J Reprod Immunol 51 (4), 294-301 (2004).

2 N.M.C., 1999. Laboratory handbook on bovine mastitis. National Mastitis Council Inc., Madison WI.

**Mr Domenico Iuso**

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**Epigenetic immaturity of mature oocytes and embryos derived from prepubertal sheep**

Aberrant genome-wide methylation of fully grown germinal vesicle stage oocytes collected from prepubertal sheep has been previously reported by our group (Ptak et al., Human Reprod, 2006). Present study aimed to determinate whether insufficient methylation persists in metaphase II oocytes from prepubertal sheep and, subsequently in embryos obtained by in vitro fertilization of prepubertal sheep oocytes. To do this oocytes obtained from slaughtered lambs and adult sheep (control) were in vitro matured and directly subjected to fixation on slides according to air dry Tarkowski's technique or, in vitro fertilized and cultured till the blastocyst stage and then similarly fixed. Subsequently the slides were UV irradiated and incubated with anti-5-methyl cytosine antibody. Regions of anti-5-methyl cytosine antibody binding were visualized by indirect immunofluorescence and the intensity of the fluorescent signal was evaluated by image measurement and analysis lab software (imal 3.5.10.d). The methylation signal of chromosomes spreads obtained from metaphase II oocytes from prepubertal sheep was significantly lower ( $P < 0.01$ ) than chromosome spreads obtained from adult sheep oocytes. Globally lower methylation signal ( $P < 0.05$ ) has been noted also in blastocysts derived from in vitro fertilization of prepubertal oocytes ( $n=30$ ) versus those obtained from control adult sheep oocytes ( $n=33$ ). These data show that aberrant methylation pattern is present in fully mature i.e. competent for fertilization oocytes from prepubertal females, and furthermore, persists even in embryos which are able to progress till the blastocyst stage. These results support the epigenetic immaturity of prepubertal oocytes and embryo, which may contribute to reduced development competence of prepubertal lamb embryos

### **Transgenic pig models for studying effects of disturbed glucose metabolism on embryonic and fetal development**

Pregnancy in type 1 and type 2 diabetes mellitus is associated with an increased rate of adverse outcomes for both the mother and the fetus. In order to study consequences of disturbed glucose metabolism on embryonic and fetal development in large animal models, we generated transgenic pig models exhibiting impaired glucose tolerance and insulin secretion. Transgenic pigs expressing a dominant-negative receptor for the incretin hormone glucose-dependent insulinotropic polypeptide (GIPR<sup>dn</sup>) in the pancreatic islets exhibit a progressive deterioration of glucose control. In 11-week-old GIPR<sup>dn</sup> transgenic pigs (n=5) oral glucose tolerance was significantly ( $p<0.05$ ) reduced, with a delay in insulin secretion as compared to controls (n=5), although the total insulin secretion capacity was not different between the two groups. In contrast, 5-month-old GIPR<sup>dn</sup> transgenic pigs exhibited markedly reduced insulin secretion in response to oral glucose challenge ( $p<0.01$ ), resulting in significantly elevated glucose levels ( $p<0.05$ ). In 11-month-old GIPR<sup>dn</sup> transgenic pigs intravenous glucose tolerance was also diminished, due to reduced insulin secretion and a severe reduction ( $p<0.01$ ) of pancreatic beta-cell mass.

A second model was established by using a mutant porcine insulin sequence (C93S) under the control of 1.3 kb insulin promoter sequences. A floxed neomycin resistance gene at the 3' end of the transgene was used as a selection marker. Transgenic founder pigs were generated by somatic cell nuclear transfer (SCNT) cloning using pools of transfected fibroblasts. Five of the six cloned piglets were transgenic, with different integration patterns in all animals examined. At the age of eight months, two of the founder pigs exhibited markedly reduced intravenous glucose tolerance. Cells from these founders are currently being used for re-cloning to further characterize the phenotypic consequences.

Both models will be used to evaluate consequences of maternal disturbed glucose metabolism on the development of embryos and fetuses and their interactions with the maternal environment.

**Dr Ammar Ali**Ali Ammar Bin Talib<sup>1,2</sup>, Pasciu V.<sup>2</sup>, Naitana S.<sup>2</sup>

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<sup>1</sup>Department of Pediatrics UCSD & Burnham Institute/Stem Cell Center, La Jolla 92037 San Diego, CA - USA, <sup>2</sup>Department of Animal Biology, University of Sassari, via Vienna no.2, 07100 Sassari – Italy**The Effect of Reduced Sperm-Oocyte Exposure Time on Ovine Embryo Production**

Semen diluents usually contain egg yolk (EY). However, bovine serum albumin (BSA) has been used in place of EY in sheep (Ali *et al.*, 2008). Reducing exposure time of oocytes to spermatozoa creates fewer reactive oxygen species, increases early cleavage, oocyte fertilization, blastocyst formation, and results in faster embryonic development with superior morphological quality (Dirnfeld *et al.*, 1999; Gianaroli *et al.*, 1996; Kattera *et al.*, 2003; Hoshi *et al.*, 2008). The aim of this study was to examine the effect of reducing gamete co-incubation time during IVF from 18-20 hr to 1, 2, or 3 hr by using frozen-thawed semen prepared from different concentrations of BSA on the subsequent embryonic development.

Sarda ewe oocytes were collected and matured for 24hr. Matured oocytes were partially denuded of cumulus cells and divided into three groups, fertilized using frozen-thawed ram semen diluted with different concentrations of BSA (single ejaculate), and incubated for varying times. Zygotes were washed, removed from wells at 1, 2, and 3 hrs post insemination (hpi) and placed in IVC. The number of expanded blastocysts/group (144-192 hpi) were recorded and vitrified.

The cleavage rate increased with gamete co-incubation time in the 10 and 15% BSA groups. There were significant increases in cleavage rates in 10 vs. 20 % BSA, and 15 vs. 20% BSA after 1hr and 2hr. The rate of blastocyst formation increased significantly with gamete incubation time. There was also a significant increase in number of blastocysts (< 168 hpi, P< 0.05) in 10% BSA from 2 to 3hr, and in 20% BSA from 1 to 2hr. The number of blastocysts was significantly (P< 0.01) greater in 20% BSA vs. 10% at 2hrs. We have concluded that: (1) spermatozoa-oocyte interaction takes place within 1h and (2) both cleavage and blastocyst rate increase linearly with gamete co-incubation time with no evidence of polyspermy.

Ali *et al.*, 2008. GEMINI 1<sup>st</sup> General Meeting Volos/ Greece. pp.18.

Hoshi *et al.*, 2008, *Reprod Dom.* 44, 634-637(4)

Dirnfeld *et al.*, 1999. *Hum Reprod* 14: 2562-4.

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## **Molecular Characterisation of Bovine Endometrium and Uterine Flushings by a LC-MS/MS Based Proteomic Approach**

An intact uterine environment is crucial for embryo development and the maintenance of pregnancy. For a comprehensive functional and biochemical characterization of this environment, the generation of an inventory of uterine proteins and protein modifications is a prerequisite. In the work presented here, we performed a qualitative proteome analysis of bovine endometrial tissue. A combination of one-dimensional-gel electrophoresis and two-dimensional liquid chromatography - mass spectrometry was applied to generate a large dataset of MS/MS spectra derived from endometrial tissue. Limiting the false discovery rate to < 1%, more than 1.500 different proteins were identified with a high confidence level. Gene ontology analysis of identified proteins revealed a broad diversity of biological and biochemical functions as well as cellular localizations.

Since uterine protein secretion is a major mechanism for the modulation of the uterine environment during estrus cycle and pregnancy, the protein composition of uterine flushings was characterized in a second approach. The combination of pre-fractionation by one-dimensional-gel electrophoresis and liquid chromatography – mass spectrometry lead to the identification of more than 350 proteins. Interestingly, a considerable fraction of proteins was below the detection limit in endometrial tissue samples, but could be detected with a high confidence level in uterine flushings.

**Prof Toni Dovenski**

Trojancanec Plamen, Petkov Vladimir, Atanasov Branko, Grizelj Juraj

Lecturer

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**Monitoring of early embryonic mortality in dairy cows using ultrasound and progesterone**

Study of maternal interaction with embryo in early stage may be improved by using ultrasonography. This tool offers opportunity for noninvasive, highly accurate monitoring of the uterine and ovarian structures. Embryonic loss during first 42 days of pregnancy presents the major problem of decreased dairy cow fertility. It has been estimated recently that early embryonic mortality presents more than 50% of pregnancy failure in cattle (mostly before day 17). The aim of this study was to observe the influence of some characteristics of ovarian structures (corpus luteum diameter and progesterone profile) as a possible cause of infertility.

For that reason Holstein-Friesian multiparous cows (n=27) were synchronized with PGF<sub>2</sub>α and inseminated after oestrus was detected. Blood sampling and transrectal ultrasonography of ovaries and uterus were performed on day 15, 21, 26 and 31 after AI with 7.5 MHz rectal probe (Aloka SSD 500 Micrus). Plasma samples were collected and analyzed for progesterone (P<sub>4</sub>) concentration by enzyme immunoassay (EIA) method on the same days.

The cows were considered pregnant if corpus luteum (CL) diameter was ≥2.5 cm on day 21 (criteria after Dovenski, 1998). Twenty cows were considered pregnant with average CL diameter of 2.58 ± 0.06 cm and P<sub>4</sub> plasma level of 3.85 ± 0.28 ng/ml respectively.

Positive pregnancy diagnosis, based upon finding of an anechoic zone in the uterine lumen and a hyperechoic conceptus inside on day 26 and 31 was found in 17 cows (85%). P<sub>4</sub> plasma level was 4.11 ± 0.47.

Differences in plasma P<sub>4</sub> level between pregnant and non-pregnant animals were statistically significant (p=0.0187) on day 15 (4.3 ± 0.35 vs. 3.11 ± 0.36 ng/ml), regardless of non-significant differences in CL diameter (2.62 ± 0.05 vs. 2.63 ± 0.97 cm) respectively. Ultrasonography could be useful tool for detection of pregnancy loss in dairy cows; however it is inefficient before day 21.

### **Efficiency of ultrasonographic survey of assisted reproduction techniques in goats**

The aim of research was to establish the importance and accuracy of the ultrasonic examination of donor and recipient goats submitted to assisted reproduction procedures.

For this reason, 17 donor goats were systematically examined by ultrasound to count the preovulatory follicles ( $\geq 5$  mm) at observed oestrus onset and to confirm occurred ovulation(s). The accuracy of ultrasound exams was measured comparing previous findings with number of *corpora lutea* (CLs) present when flushing was performed.

On the other hand, 14 recipient goat ovaries were monitored by ultrasound to assess: the number of dominant follicles ( $\geq 5$  mm) present during oestrus, occurred ovulation as well as echogenic quality of CLs before semylaparoscopic embryotransfer was carried out.

The total relative undervalue of ultrasound prediction of ovulation was found to be 38.37%. The response of 9 out of 14 (64%) recipient goats, after standard hormonal protocol was applied, was adequate, what means that at most two dominant follicles, timely occurred ovulation and good echogenic quality of respective CLs was found.

The ultrasonic examination of donor and recipient goats is appreciable tool to follow up follicular dynamics, success of superovulation procedures and to confirm the ovulation and echogenic CLs quality, as well as to predict the ovulatory rate.

**Ms Anna Groebner**AE Groebner<sup>1</sup>, K Schulke<sup>1</sup>, H Reichenbach<sup>2</sup>, HHD Meyer<sup>1</sup>, SE Ulbrich<sup>1</sup>

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<sup>1)</sup> *Physiology Weihenstephan, Technical University of Munich, Germany*<sup>2)</sup> *Bavarian State Research Center for Agriculture, Institute of Animal Breeding, Grub, Germany***Studying immunologically relevant genes in the bovine endometrium during the window of implantation using a dynamic gene expression array**

How is the immune system modulated to establish a successful pregnancy in the bovine endometrium during the preimplantation phase? As the semi-allogenic embryo is repeatedly accepted by the mother, the aim of this study was to gain insights into the immunological status during the window of implantation. Uteri of Simmental heifers were flushed post mortem during the oestrus cycle and the preimplantation phase in the bovine endometrium for the recovery of embryos and the sampling of ipsilateral endometrium to analyse the mRNA expression of immune related genes. A high throughput gene expression platform was used based on microfluidic dynamic arrays (Fluidigm® 96.96 Dynamic Array). This allows 9,216 simultaneous real time PCR gene expression measurements per chip. In a trial experiment setup, the mRNA expression of 32 genes in 94 preamplified endometrial and embryonic samples each was measured with three replicates on a single chip. We obtained an average standard deviation of 0.1616 and a chip to chip correlation of >0.99 for all three replicates. The platform thus allows a large scale analysis of real time PCR assays with a high dynamic range and sensitivity. Interestingly, indoleamine 2,3-dioxygenase 1 (IDO1) was significantly 29-fold ( $p < 0.001$ ) more abundant in day 18 pregnant vs. non-pregnant control endometrium. IDO1 catalyzes the cleavage of pyrrol rings to prevent deleterious T cell activation which is dependent on tryptophan availability. Additionally, we found a 1.6-fold ( $p = 0.003$ ) increased expression of a bovine leucocyte antigen (BoLA) MHC I gene (similar to human HLA-G) in day 18 pregnant endometrium compared to cyclic controls, whereas BoLA was 3,000-fold lower expressed in day 18 trophoblasts. In conclusion, inhibition of non-self recognition and avoidance of detrimental leukocyte activation may be possible mechanisms at the feto-maternal interface used to allow embryo development and trophoblast attachment.

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**Prof Sabine Koelle**

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**Behaviour of spermatozoa in the female genital tract – new insights in physiology and influence of semen diluents gained by digital videomicroscopy**

Mammalian spermatozoa form a sperm reservoir in the ampulla enabling them to stay vital for days up to months. Using digital videomicroscopy in the bovine we were able to show that only vital spermatozoa bind to the oviductal epithelium. The number of bound spermatozoa is significantly decreased in bulls with low fertility as compared to bulls with high fertility. Bulls with beginning inflammation of joints, which did not yet show clinical signs or alterations in standard sperm assessment, revealed distinct reduction of sperm binding to the oviductal epithelium. When comparing behaviour of spermatozoa after cryopreservation in semen diluents with and without egg yolk, it became obvious that the post-thaw sperm motility in the oviduct as well as binding to the oviductal epithelium was reduced in spermatozoa diluted in semen diluent containing egg yolk as compared to semen diluent without egg yolk. However, this effect was reversed as soon as the semen diluent was diluted with oviductal fluid. In order to create a new and sensitive method for sperm assessment, a sperm binding assay on coated slides containing fucose was successfully established.

Our results imply that sperm binding to oviductal epithelium is correlated to fertility. The determination of the binding capacity to the oviductal epithelium is a highly sensitive method to assess the quality of an ejaculate. Thus, the new sperm binding assay may provide valuable additional information for the prognosis of male fertility.

### **Tissue-Specific Selection of Reference Genes is Required for Expression Studies in the Mouse Model of Maternal Protein Undernutrition**

Studies into the effects of maternal nutrition have established a significant role for sub-optimal diet during gestation in the establishment of long-term phenotypic change and increased disease risk in offspring. In order to elucidate the route through which environmental sensitivity manifests itself in phenotypic change, investigation into the molecular characteristics of offspring following sub-optimal maternal nutrition during gestation now has become the focus of many studies. However, the likely role of key cellular processes such as metabolism, DNA synthesis and transcription in the response to maternal undernutrition raises the question of whether those genes typically used as reference constants in gene expression studies are suitable in nutritional manipulations.

Using our mouse model of maternal protein undernutrition, we have investigated the stability of seven commonly used reference genes (*18s*, *Hprt1*, *Pgk1*, *Ppib*, *Sdha*, *Tbp* and *Tuba1*) in a variety of offspring tissues including liver, kidney, heart, retro-peritoneal and inter-scapular fat, placenta and yolk sac. Reference gene expression was not found to be affected by dietary treatment, indicating that these genes are suitable for normalisation of gene expression in the studied tissues. However, we found that the most stable genes differed in a tissue-specific manner, and furthermore that the relative expression levels of some reference genes differ significantly between tissues of similar function. For example, *Sdha* and *Pgk1* transcript levels were significantly lower in retro-peritoneal than inter-scapular fat tissue (62% and 77% lower, respectively; both  $p > 0.05$ ). This suggests that specific attention should be paid to reference gene selection for cross-tissue comparisons.

In summary, we find that although our selected reference genes are all highly stable within this dietary model, stability varies in a tissue-specific manner, and recommend that gene expression studies should be tailored to suit the tissue, or tissues, of interest.

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**Serum anti-endometrial antibodies in infertile women are a potential risk factor for implantation failure**

Female infertility patients with diverse aetiologies characteristically show an increased production of autoantibodies. Although anti-endometrial antibodies (AEA) are mainly associated with endometriosis, they may also exist in other infertile women. AEA may hamper female fertility by various mechanisms, since the presence of AEA has been demonstrated in patients with ovulatory disorders and tubal obstructions, as well as in patients with decreased endometrial receptivity and recurrent implantation failure. AEA recognise a wide range of endometrial antigens between molecular weight range 15-170 kDa. However, the nature of most of these cognate antigens is unclear. In our study, 1- and 2-dimensional immunoblot analyses of sera from patients with endometriosis and tubal factor infertility (TFI) demonstrated the presence of IgA and IgG AEA to various antigens at molecular weights ranging from 10 to 200 kDa. Differences were detected in certain anti-endometrial antibody reactions between the patient groups, and particular AEA were associated with *in vitro* fertilization (IVF) implantation failure. IgA AEA to a 47 kDa protein were more prevalent in TFI patients and were associated with unsuccessful IVF treatments. This antigen was subsequently identified as  $\alpha$ -enolase. In conclusion, our data demonstrate differences in humoral immune responses in infertile endometriosis and TFI patients and a negative association between the occurrence of certain AEA and IVF pregnancy outcomes. Consequently, determination of the presence and spectra of AEA in patients undergoing IVF may be useful to predict pregnancy outcomes in these patients.

**Dr Kristy Dremmers**

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**Ewes heterozygous for the Inverdale mutation (Fec X<sup>l</sup>) show greater ovulation rate response following increased pre-ovulation nutrition compared with non-carriers of the gene**

This study aimed to determine whether ewes heterozygous (I+) for the Inverdale mutation with a high natural ovulation rate (OR), show similar sensitivity to nutritional manipulation as non-carriers (++). This naturally-occurring mutation of the bone morphogenetic protein-15 (BMP15) gene leads to increased OR in heterozygotes. Increasing nutrition prior to mating results in increased OR but whether this effect occurs in ewes with high natural OR is unknown.

In 2008, 162 ewes ( $62.8 \pm 0.9$  kg; 78 I+, 84 ++) and in 2009, 177 ewes ( $63.7 \pm 0.8$  kg; 89 I+, 88 ++), were assigned into two nutritional groups, High or Control. The High group were given ad-lib (3.5 kg DM/ewe/day) and the Controls maintenance (1.2 kg DM/ewe/day) pasture allowances for 6 weeks with live-weight monitored through the trial. Oestrus was synchronised (12 days CIDR-G) at the end of the feeding period, and OR measured by laparoscopy 8 days later.

The High group increased in weight compared with Controls (+3.9 kg 2008; +8.0 kg 2009; both  $P < 0.01$ ) which was accompanied by an increase in average OR (High vs. Control:  $2.8 \pm 0.1$  vs.  $2.5 \pm 0.1$ , 2008,  $P = 0.02$ ;  $2.9 \pm 0.1$  vs.  $2.2 \pm 0.1$ , 2009;  $P < 0.01$ ). This OR effect was greater in 2009 with the larger weight gain. As well as higher OR in I+ ewes ( $+1.26 \pm 0.13$ ,  $P < 0.01$ ), there was a genotype/group interaction ( $P < 0.001$ ), with I+ ewes in the High group showing a greater increase in OR than ++ ewes (High vs. Control: I+  $3.48 \pm 0.1$  vs.  $2.70 \pm 0.1$ ; ++  $2.23 \pm 0.1$  vs.  $2.03 \pm 0.1$ ).

This study shows that I+ ewes respond to increased pre-mating nutrition with a larger increase in OR than non-carriers. This greater sensitivity could indicate an interaction between BMP15 levels and nutritional signals in the follicle to control OR in sheep.

### **Acute maternal protein overnutrition alters embryonic cell-lineage allocation, signalling activity and biosynthesis levels**

Maternal malnutrition during fetal development alters metabolic processes involved in growth regulation of the developing fetus resulting in offspring obesity and metabolic diseases. Such phenotypic changes can be mediated by mechanisms for sensing energy status and adapting biosynthesis levels such as mTOR or AMPK (2,3). We have already established that maternal protein undernutrition *exclusively during the preimplantation period* can induce similar postnatal phenotypes (1). Measurable changes are detectable by the blastocyst stage when cell lineage divergence into inner cell mass (ICM, embryo proper) and trophectoderm (TE, placental lineages) emerges, coinciding with major metabolic transitions. For example, blastocyst cell lineage allocation and mTOR signalling are altered, collectively suggesting compensatory mechanisms whereby energy status and signalling responses to nutrition maintain anabolic activity and growth potential of the conceptus. However, we know little about *directionality* of the responses to nutrition levels. Here, we compare cell allocation, protein biosynthesis and energy/stress status indicated by AMPK $\beta$ 1 distribution in blastocysts derived from mothers fed a control (18% casein) or elevated (30% casein, HPD) protein diet during the preimplantation period. In HPD-blastocysts, the ICM was reduced ( $p < 0.001$ ) whilst TE and total cells were maintained thus directing cells towards TE ( $p < 0.001$ ) just prior to implantation (d3.75). Such blastocysts synthesised more protein compared to controls (50% upregulation;  $p < 0.001$ ) up to 12hrs after removal from the challenge. AMPK $\beta$ 1 as indicator of cellular stress was found increased in TE nuclei in HPD blastocysts. Altogether, this suggests that early embryos can raise their anabolic metabolism in plentiful conditions. However, maternal protein overnutrition may cause cellular stress diverting blastomeres away from the ICM lineage towards the metabolically more active TE just prior to implantation. Such lineage diversion may help the embryo cope better with adverse intrauterine environments by tightly controlling the ICM environment and ensuring embryonic-maternal cross-talk sufficient for implantation.

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**Mrs Sünje Fischer**

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**Adiponectin influences the glucose metabolism  
of the preimplantation embryo of the rabbit**

Since the discovery of adipokines, the adipose tissue is no longer considered to be an inactive fat storage. It is secreting a variety of bioactive molecules, which regulate body metabolism and energy homeostasis. Recent studies point to a role of adipokines like leptin and adiponectin in reproduction.

Adiponectin is a 26 kDa peptide hormone which has various functions in lipid and carbohydrate metabolism. It is well described that low plasma levels of adiponectin are associated with metabolic disorders. It acts via its specific receptor (adipoR), which exists in two isoforms, adipoR1 and adipoR2. It triggers metabolic effects through the AMP-activated protein kinase (AMPK), which is a master regulator in glucose and lipid metabolism.

Immunohistochemistry revealed that adiponectin and its receptors are expressed in the trophoblast and the embryonic disk of day 6 *p.c.* rabbit blastocysts.

An *in vitro* supplementation of adiponectin (1mM) to day 6 *p.c.* rabbit blastocysts enhanced AMPK phosphorylation and decreased the transcript numbers of the metabolic target gene phosphoenolpyruvate carboxykinase (PEPCK). Furthermore adiponectin caused the translocation of the glucose transporter 4 (GLUT4) and consequently an increase in glucose uptake.

By using a specific inhibitor of the AMPK, compound C (10 $\mu$ M), the decreased transcript numbers of PEPCK could be restored. This means adiponectin acts through AMPK in rabbit blastocysts.

All these results lead to the conclusion, that adiponectin influences the glucose metabolism of rabbit blastocysts via the phosphorylation of AMPK and by down-regulation of PEPCK transcript numbers. This, in turn, results in a decrease of gluconeogenesis. Furthermore, adiponectin prompts the glucose uptake via an intensified GLUT4 translocation and this may result in an increased glycolysis.

The obvious influence of adiponectin on the glucose metabolism of rabbit blastocyst may be of specific interest in pathophysiological situations such as obesity during pregnancy.

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<sup>1</sup>Facultad Veterinaria. UCM. Madrid, Spain; <sup>2</sup>ETSIA. UPM. Madrid, Spain**Influence on body condition and ovarian status of two reproductive rhythms in primiparous non-weaned rabbit does**

This work assesses the body reserves and ovarian status of 24 primiparous lactating females (8-10 kits per doe) on Day 11 and 25 *post-partum* (pp) corresponding to usual artificial insemination times. Live body weight (LBW), estimated body composition and blood samples were obtained and ovaries recovered for histological and *in vitro* oocyte maturation studies. Body composition measured by electric bioimpedance significantly varied between groups in relation to lipids ( $18.1 \pm 0.7$  vs.  $12.3 \pm 1.0\%$ ;  $P < 0.001$ ), energy ( $1177 \pm 25$  vs.  $96 \pm 44$  KJ/100g;  $P < 0.001$ ) and protein contents ( $62.1 \pm 0.5$  vs.  $66.2 \pm 0.9\%$ ;  $P < 0.001$ ) on Day 11 and 25 pp, respectively. Besides, animals on Day 11 pp showed slightly higher LBW ( $4095 \pm 75$ g vs.  $3561 \pm 353$ g;  $P = 0.16$ ). Similar serum oestradiol concentrations, measured by ELISA, were found in both groups ( $44.3 \pm 2.9$  vs.  $57.8 \pm 18.9$  pg/ml, respectively) but a tendency to show higher progesterone concentrations were observed on Day 25 pp group ( $0.8 \pm 0.3$  vs.  $2.5 \pm 1.1$  ng/ml;  $P = 0.13$ ). The pp day considered did not affect the mean of primordial ( $73.9 \pm 2.3$  vs.  $72.4 \pm 3.1\%$ ), primary ( $11.2 \pm 1.3$  vs.  $10.2 \pm 1.5\%$ ) and secondary follicles ( $9.3 \pm 0.9$  vs.  $9.3 \pm 1.7\%$ ) observed but those antral were slightly higher on Day 25 pp ( $5.6 \pm 0.9$  vs.  $8.0 \pm 1.7\%$ ;  $P = 0.19$ ). Percentage of healthy follicles assessed by TUNEL were slightly higher on Day 11 pp ( $55.2 \pm 5.6$  vs.  $44.5 \pm 4.7\%$ ;  $P = 0.17$ ). Nuclear matured oocytes measured by metaphase II rate were similar for both experimental groups (67.2 vs. 66.6%). In conclusion, primiparous does nursing more than 8 kits reach to day 25 pp with low energy reserves although ovarian features and serum steroid concentrations did not seem to be severely impaired.

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<sup>1</sup>Facultad Veterinaria. UCM. Madrid, Spain; <sup>2</sup>ETSIA. UPM. Madrid, Spain.**Effect of energy supply during short time on ovarian status and oocyte maturation of primiparous lactating rabbit does**

The goal of this work was to study the influence on ovarian status and oocyte maturation of two oestrus synchronization methods in primiparous lactating rabbits. Ovaries of 12 animals at day 11 post-partum were recovered 48h after administration of 25 IU of equine chorionic gonadotrophin (eCG; n=6) or 4 days after supply of 2% propylenglycol in water (n=6). In each animal, one ovary was allocated for histological studies and the other one for *in vitro* oocyte maturation evaluation. Serum oestradiol concentrations, measured by ELISA, were similar for both treatments (38.7±4.9 vs. 49.8±1.4 pg/ml). Also, population of follicles ≥ 1mm at the ovarian surface (16.3±0.6 vs. 15.8±1.8), as well as number of primordial (70.9±3.4 vs. 77.0±3.0%), primary (13.1±2.0 vs. 9.2±1.5%), secondary (9.9±1.1 vs. 8.6±1.5%) and antral follicles (6.0±1.5 vs. 5.2±1.0%) were not different in ovaries of eCG and flushing treated animals. Neither apoptotic follicles index (12.1±6.0 vs. 29.5±7.2%) assessed by TUNEL. Nuclear and cytoplasmically matured oocytes measured by metaphase II rate (64.1 vs. 68.0%) and complete cortical granules migration index (14.7 vs. 18.1%) were similar for both experimental groups. However, a higher percentage of cytoplasmically inmatured oocytes was found in eCG treated animals (55.8 vs. 18.1%; P<0.001) and consequently, the number of oocytes partially matured was lower in that group (14.7 vs. 36.3%; P<0.05). In conclusion, flushing as 'focus feeding' in primiparous lactating rabbits did not affect reproductive ovarian outcome.

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**Subclinical endometritis as cause of changed endometrial environment in cows**

Currently, the endometrial environment and subclinical endometritis are being discussed as an important cause of low fertilization rates in inseminated cows.

The aim of the study was to evaluate the prevalence and the nature of subclinical endometritis during the service period in dairy cows.

Using the cytobrush technique, endometrial epithelium samples were collected in 2 herds from 116 cows without clinical signs of endometritis at 50-70 days post partum. The smears were stained by Papanicolaou staining and a total of 100 cells were counted to determine the percentage of polymorphonuclear leucocytes (PMNs). A threshold of 10 % PMNs was used as the definition of subclinical endometritis. Additionally, the concentration of  $\text{PGF}_{2\alpha}$ , PGE and  $\text{TNF}\alpha$  in uterine secretions was measured by EIA in cows grouped according to the percentage of PMNs (> 10%, 20%, 40%). The endometritis rate differed between the herds (36.3% vs 15.2%) The bacteriological background of this phenomenon was found only in 33% of these cases in herd A and 14,9% in herd B. Concentrations of  $\text{PGF}_{2\alpha}$  and  $\text{TNF}\alpha$  were increased in cows with endometritis proportional to the percentage of PMNs (up to 0.35 ng/ml and 1800 pg/ml, respectively). On the contrary, the concentration of PGE was diminished in cows with endometritis. The inseminations index was significantly higher in cows with increased PMNs number than in other animals (herd A – 2.4 vs 1.7, herd B 1.9 vs 1.7, respectively).

Our study showed different rate of subclinical endometritis in both herds, accompanied by lowered fertility. The dysregulation of prostaglandins F and E, as well as of  $\text{TNF}\alpha$  seems to have crucial consequences for the embryo.

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**Maternal protein undernutrition in mouse alters DNA methyltransferase transcript expression**

While the adverse effect of a sub-optimal maternal diet on the postnatal health and disease risk of offspring has been reported extensively, the precise mechanisms underlying the establishment of programmed response during early development are as yet poorly understood. Epigenetic changes during early development are an attractive candidate mechanism for establishing the long-term effects of sub-optimal maternal diet in offspring. The most studied epigenetic modification, DNA methylation, has been shown previously to be altered in rat offspring in response to low protein maternal diet, likely due to altered methyl cycle parameters.

Since DNA methyltransferases (DNMTs) are responsible for the establishment and maintenance of DNA methylation patterns within the genome, this study aimed to examine DNMT expression in the low protein diet *mouse* model, both in embryonic, extra-embryonic and adult tissues, to determine whether epigenetic changes may be associated with the development of the reported postnatal phenotypes.

Dams were fed either a control diet throughout gestation (normal protein diet; NPD), a low protein diet throughout gestation (LPD), or low protein for the preimplantation period (to 3.5dpc) followed by normal protein diet for the remainder of gestation (Emb-LPD). Relative transcript expression was examined by RT-qPCR using intron-spanning primer sets, and normalised to reference gene expression levels using geNorm. Tissue- and sex-specific differences in DNMT transcript expression were observed in response to maternal low protein diet, both in adult tissues and in tissues from day 17.5 conceptuses. For example, in adult kidney the maintenance methyltransferase, DNMT1, was upregulated in LPD females ( $P<0.05$ ), and at day 17.5 both DNMT1 and the *de novo* methyltransferase, DNMT3b, were downregulated in LPD placenta ( $P<0.05$ ).

The changes we see in DNMT expression may lead to downstream changes in gene expression in response to the low protein diet, resulting in the reported phenotypic consequences of maternal protein undernutrition.

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**The fetal and neonatal effects of periconceptual hyperglycemia using a rabbit model**

The aim of this study was to analyze the effects of maternal hyperglycaemia during the periconceptual period on fetal and neonatal development using a rabbit model.

Diabetes was induced with alloxan in adult female rabbits one week prior to mating. Glycemia was maintained between 3 and 5 g/l with 2 subcutaneous insulin injections per day.

In a first experiment, embryos of diabetic (D, n=20 embryos) and control (C, n=21) groups were collected on D4 post conception and transferred to 6 non diabetic recipients (3 for each group). Fetal and placental development were monitored by ultrasound. At birth, litters were equilibrated by number and sex. Bodyweight and adiposity (by densitometry) were monitored until sacrifice at one month of age. In a second experiment, D4 embryos from D (n=11) and C (n=13) does were transferred respectively to the right and the left horn of 3 female recipients. Recipients were euthanised on Day 28.

In the first experiment, fetal development as assessed by ultrasound was not significantly different between D and C groups. Birthweight, however, was significantly reduced in male but not female offspring from the D group ( $p<0.05$ ). Male pups caught up and there was not significant difference in weight after 7 days. At one month of age, there were no statistical differences between D and C groups for adiposity and plasma insulin and leptin concentrations. In contrast, fasting plasma glucose was significantly lower in male D offspring ( $p<0.01$ ). Moreover, kidney weight was significantly lower in male D offspring ( $p<0.05$ ). In the second experiment, although fetal weight was no statistically different between D and C groups, brain weight was significantly lower in fetuses exposed to maternal hyperglycemia ( $p<0.05$ ).

These data suggest that maternal hyperglycemia during the periconceptual period affects glucose metabolism and organ development in offspring, with a sexual dimorphism. Further work is being pursued to study longer term effects.

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**Genetic and epigenetic alterations induced by polychlorinated biphenyls in sheep embryonic and extraembryonic cells *in vitro***

Polychlorinated biphenyls (PCBs) are toxic contaminants globally distributed in the environment. Prenatal exposures to PCBs through transplacental transport has been reported to cause adverse effects on the foetus. Little is known about times and mechanism of action in the embryo. In this study cultured sheep embryonic fibroblast and extraembryonic amniocytes were exposed to PCBs at a low concentration (1 µg/ml) for 15 and 30 days. Sister Chromatid Exchanges (SCEs) was elected as an analytical tool to evaluate genotoxic effects of PCBs. PCBs significantly ( $P < 0.001$ ) enhance the frequency of SCEs in both embryonic (PCB  $5,75 \pm 2,33$  vs CTRL  $3,6 \pm 1,8$ ) and extraembryonic cells (PCB  $6,97 \pm 2,9$  vs CTRL  $4,36 \pm 2,15$ ) after 15 days of treatment. In addition, we demonstrate that a longer exposition (30 days of treatment) increases the number of SCEs/cell in a time-dependent manner. Furthermore, we hypotized that epigenetic alterations can occurs as consequence of DNA damage due to PCBs. Genome-wide methylation has been analyzed using 5-Methyl-cytosine antibody incorporation on metaphasic chromosomes. Our observation does not indicate any significant differences in methylation patterns between PCBs treated and control groups. Probably a different DNA-methylation status can be observed using a more sensitive analysis. In conclusion, this work prompts further investigation about the correlation between DNA-damage and epigenetic status in embryos exposed to PCBs

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### **Effects of diabetes type 1 on the preimplantation uterine milieu in rabbits**

Increasing numbers of young women are affected by diabetes. Diabetes is not only correlated with reduced fertility, but also with a higher risk for spontaneous abortions and congenital malformations, indicating the close relationship between maternal nutrition and metabolism and embryo development.

To investigate diabetogenous effects on the maternal uterine milieu and embryo development during early pregnancy, we developed a pregnant hyperglycaemic rabbit (PHR) model. Diabetes is induced by a single alloxan treatment 10 days before mating. After alloxan treatment the rabbits rapidly develop a hyperglycaemic blood glucose concentration of  $\geq 20$  mM. Today we report on uterine secretions and endometrial transcripts in diabetic and control rabbits on day 6 *post coitum*.

In the uterine secretions the glucose concentration was increased by 3.5fold from approx. 0.5mM to 1.7mM. The three aliphatic branched chain amino acids (BCAA) leucine, isoleucine and valine were increased. Several changes in the transcriptional levels of IGF family genes were noticed. The mRNA for IGF1 and 2 was increased in diabetic uteri. While the amount of the IGF1 receptor (IGF1R) transcript was reduced the IGF2R transcript was increased. There was no effect on the transcriptional level of the insulin receptor (IR). The IR was localized in the luminal epithelial cells and in smooth muscle cells.

Our findings show significant changes in the composition of uterine secretions and in the transcriptional level of genes involved in the IGF receptor system. These changes could be one explanation for the observed dysfunctions in diabetic women.

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**Elevated offspring growth induced by maternal protein undernutrition exclusive during preimplantation development is associated with altered signalling through the mammalian target of rapamycin (mTOR) pathway in mice**

The mammalian target of rapamycin (mTOR) coordinates cell growth and cell cycle progression in response to nutrients (amino acids), energy (ATP:ADP) and growth factors via the phosphorylation of down stream targets including 4EBP1 and the 70S ribosomal protein S6. Phosphorylation of S6 and 4E-BP1 results in ribosome biogenesis, protein translation, increased cell mass and the translation of key growth-promoting proteins such as c-Myc and cyclin D1. Here we investigate whether altered signalling patterns through the mTOR pathway may associate with an enhanced growth phenotype observed in offspring mice from females fed a low protein diet exclusively during preimplantation development.

Following mating, female MF-1 mice were assigned to one of the following dietary treatments (a) normal protein diet of 18% casein (NPD), (b) isocaloric low protein diet of 9 % casein (LPD), or (c) LPD for the preimplantation period (3.5 days) then switched to NPD for the remainder of gestation (Emb-LPD).

Embryonic, fetal and adult tissues were collected and the levels of total and phosphorylated 4EBP1 and S6 were determined using quantitative western blotting. Levels of phosphorylated S6 and the ratio of phosphorylated:total S6 were lower in blastocysts from Emb-LPD mothers. Levels of total S6 were elevated in adult livers of Emb-LPD mice whilst phosphorylated S6 levels were decreased in adult heart tissue from LPD mice when compared to controls. Reduced levels of phosphorylated 4EBP-1 were observed in LPD and Emb-LPD adult liver tissue when compared to controls.

These data demonstrate that maternal undernutrition during discrete windows of gestation result in significant changes in the levels of key down-stream mTOR pathway mediators. These changes appear to initiate in the preimplantation embryo, and perpetuate into adult life. However, not all tissues and pathways were equally affected suggesting developmental and tissue-specific responses in the regulation of protein synthesis, cell division and translational control.

**Prof Christine Wrenzycki**

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**Dietary CLA supplementation affects developmental competence of oocytes in lactating dairy cows**

Dietary conjugated linoleic acid (CLA) and fat supplements may benefit reproductive outcome by improving energy intake and reducing the extent of negative energy balance. Preliminary data show that a high CLA diet fed to animals after calving might improve the developmental potential of oocytes recovered from these animals via repeated OPU sessions (Höffmann et al., 2008).

The objective of this study was to examine the effect of dietary CLA supplementation on the developmental competence of oocytes from Holstein cows. Animals fed a silage-based diet supplemented with either no or high (100 g/day) CLA were slaughtered at day 45 (n=5 animals per group) and day 105 (n=3-4 animals per group) after calving. Cumulus-oocyte-complexes (COC) were harvested from the ovaries of each animal at each time point and subjected to a standard IVP protocol. Cleavage rates at day 3 and developmental rates at day 7 were determined.

The mean number of COC used for IVP did not differ between the animals from each group [d 45: 18 (no CLA), 15.3 (high CLA); d 105: 32 (no CLA), 26 (high CLA)]. Cleavage rates (%) were similar between the oocytes collected from animals fed the different diets at each time point examined [d 45: 26.7 (no CLA), 37.7 (high CLA); d 105: 29.4 (no CLA), 37.2 (high CLA), respectively]. Developmental rates at day 7 (%) were significantly increased from oocytes collected from animals fed the high CLA diet compared to oocytes from the group fed no CLA [d 45: 10.0 (no CLA), 21.3 (high CLA); d 105: 8.1 (no CLA), 21.8 (high CLA), respectively].

In conclusion, a high CLA diet fed to lactating dairy cows seems to improve the developmental potential of oocytes recovered from these animals.

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**Expression of HOX genes and their cofactors during the first differentiation steps of bovine and murine embryos**

Homeotic (*Hox*) genes encode transcription factors that play a crucial role in patterning the body plan in the developing embryo. Though their functions have been well established from the gastrulation stage on, previous studies have provided evidence that some *Hox* genes might be expressed during compaction and blastocoele formation and therefore play a role in very early embryogenesis.

The purpose of this study is to catalogue the expression pattern of *HOX* genes and their cofactors, *MEIS*, *PBX*, and *PREP*, during the first differentiation steps in bovine and murine embryos, so during the transition from morula to blastocyst stages.

Using primers targeting conserved domains, we amplified and sequenced 294 clones from pools of 60 *in vitro* produced bovine embryos and 60 *in vitro* cultured murine embryos, collected at morulae, compact morulae and blastocyst stages.

Until now, 20 *HOX*, 2 *MEIS*, 2 *PBX* and 1 *PREP* genes were found expressed during the first differentiation steps in the bovine embryo, while 2 *Hox*, 2 *Meis*, 2 *Pbx* and 1 *Prep* genes are transcribed in murine embryos. All members of three paralog groups (2, 4 and 7) are expressed in bovine embryos, whereas only one paralog group (7) is totally expressed in murine embryos. Finally, both animal models share a similar *MEIS* and *PREP* family expression profile, as well as a similar expression for *HOXD4*, *HOXD9* and *PBX3* genes.

As conclusion, this work shows evidence for transcription of several *HOX*, *MEIS*, *PBX* and *PREP* genes after the Maternal to Embryonic Transition, during the first steps of differentiation, in the two embryonic models. Further research will be needed to investigate the spatio-temporal expression of these genes, as well as their functional role in early embryo differentiation.

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