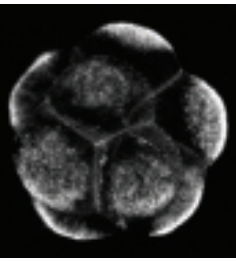

PERICONCEPTIONAL
DEVELOPMENTAL
PROGRAMMING

An early origin of disease, mediated through epigenetics or metabolic signalling

Batsheva de Rothschild Seminar on:

PERICONCEPTIONAL DEVELOPMENTAL PROGRAMMING

Proceeding of GEMINI
Group III Workshop

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Jerusalem, Israel
31st May - 3rd June, 2011

cost GEMINI

Editors: Yael Heifetz, Tom Fleming, Pascale Chavatte-Palmer

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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.

Web: www.cost.esf.org

WELCOME TO JERUSALEM

Local Host welcome

"For out of Zion shall go forth the law and the word of the Lord from Jerusalem" (ISAIAH 2, 3)

It is our pleasure to have you GEMINI members and participants of the Periconceptional Developmental Programming workshop here on Mount Scopus from which you can see the "Golden Dome" and "El-Aqsa" mosques, among the holiest points for Muslims, the Judean desert where Christianity was born, and the wall of the demolished Jewish temple. This is the city of Jerusalem – the intersection of the Three Monotheist religions that generates so much energy, sometimes ends up in tragic conflicts, but many other times provides reasons for human existence and strengthens unity and collaboration. Jerusalem is the largest city in Israel that includes a Jewish majority and around one third Palestinians at the eastern part of the city. This is also an opportunity to tell you that the Hebrew University, where the meeting will take place, is the first university in Israel, established in 1925 on Mount Scopus. We, the organising committee, hope you will find this place exciting and insightful.

The Periconceptional Developmental Programming Workshop brings together junior and established world leading scientists from Europe and Israel; from academic, governmental, and clinical sectors; using different animal models a wide range of experimental approaches to discuss recent advances and consider present challenges in understanding Periconceptional Developmental Programming. The invited speakers will highlight the most up-to-date scientific knowledge and improvements in our understanding of the relationships between epigenetic processes and environmental factors, such as maternal nutrition, and discuss the gaps in our knowledge that remain to be filled. We aim to push the frontiers of knowledge and to provide some answers to current and future challenges we face in this emerging field.

The Periconceptional Developmental Programming Workshop could not have happened without the generous support of **The Batsheva de Rothschild Foundation**, **COST**, **Society for Reproduction and Fertility** and **The Hebrew University**.

I would like also to thank the organising committee, Tom Fleming, Pascale Chavatte-Palmer and Nava Dekel for their professional help, support and the stimulating discussions about the content of the workshop. Sincere thanks go also to Lior David for his great help with the epigenetics session.

I am thankful to Roni Friedman, the dean of The Robert H. Smith Faculty of Agriculture, Food and Environment of the Hebrew University, Sharon Shafir, the head of the Entomology Department and the Entomology Department for their support. Special thanks also go to all the members of my laboratory for their support and help.

I would like in particular to thank Pnina Perlis for her essential and inspiring help in organising the workshop and Sara Gottliebsen for her professional support in administrating this workshop.

Finally, many thanks to the speakers, presenters of oral communications or posters; we believe that the exchange of information and discussion will provide a unique opportunity to learn significant new advances in the field.

Best wishes,

Yael Heifetz
Local workshop Organiser

PERICONCEPTIONAL DEVELOPMENTAL PROGRAMMING WORKSHOP

JERUSALEM, ISRAEL

31st May – 3rd June 2011

Programme

Tuesday 31st May 2011

14:30 **Registration & Programme Collection & Poster Setup**

15:15-16:00 **Reception**

16:00-16:20 **Welcome and opening remarks** - Dr Yael Heifetz
GEMINI vice chairman opening: Prof Fulvio Gandolfi
Opening by the leader of WGIII: Prof Tom Fleming

16:20-16:25 **Keynote session -** Moderators: Prof Neri Laufer & Dr Pascale Chavatte-Palmer

16:25-17:25 **Prof Howard Cedar, Hebrew University, Jerusalem, Israel**
Programming DNA methylation during development

17:25-18:15 **Prof Tom Fleming, University of Southampton, UK**
Protein undernutrition during the mouse periconceptional period alters embryogenesis, placentation and disease risk into adulthood

18:15-19:30 **Poster session & refreshments**

19:30-20:30 Dinner

20:30-22:30 **Local tour**

Wednesday 1st June 2011

07:00-08:30 Breakfast

08:30-08:35 **Morning session** - Moderators: Prof Tom Fleming & Dr Pascale Chavatte-Palmer

08:35-09:10 **Prof Kevin Sinclair, University of Nottingham, UK**
One-carbon metabolism and epigenetic programming in the oocyte and pre-implantation embryo

09:10-09:45 **Prof Nick Macklon, University of Southampton, UK**
Maternal – embryo interactions regulating success of human ART and fertility

09:45-10:20 **Prof Bernd Fischer, Martin Luther University, Germany**
Periconceptional developmental programming: Adverse effects of maternal diabetes mellitus on embryo metabolism

10:20-10:50 Coffee / Tea Break

Short communications - Moderators: Prof Adam Ziecik & Prof Joseph Orly

10:50-11:10 **Dr Francesca Mossa, University College Dublin, Ireland**
Impact of undernutrition on circulating testosterone and cortisol concentrations during pregnancy of cattle

11:10-11:30 **Dr Cristina Ovilo, INIA, Spain**
Placental gene expression and relationships with maternal reproductive features in an obese porcine breed

11:30-11:50 **Prof Dariusz Skarzynski, Institute of Animal Reproduction and Food Research of PAS, Olsztyn, Poland**
Can phytoestrogenes disrupt endocrine regulations in the equine uterus?

11:50-12:10 **Mr John Twigt, Erasmus Medical Centre Rotterdam, The Netherlands**
The preconception diet is associated with the chance of ongoing pregnancy in women undergoing IVF/ICSI treatment

12:10-12:30 **Dr Susanne Ulbrich, Technical University of Munich, Germany**
Do placental abnormalities and fetal overgrowth manifest prior to implantation through imbalances in intrauterine nutrient supply with amino acids?

12:30-13:00 **Discussion** - Moderators: Prof. Tom Fleming, Dr. Pascale Chavatte-Palmer, Adam Ziecik & Prof Joseph Orly

13:00-14:00 Lunch

14:00-15:30 **Poster session**

15:30-15:35 **Afternoon session** - Epigenetics mediates the response of cells and embryos to the environment. Moderators: **Dr Lior David** & **Dr Yael Heifetz**

15:35-16:10 **Prof Nir Ohad, Tel-Aviv University, Israel**
Epigenetic regulation of plant reproduction and development

16:10-16:45 **Dr Yoav Soen, Weizmann Institute of Science, Rehovot, Israel**
Plasticity of gene regulation and the diversification of developmental programs

16:45-17:20 **Dr Lior David, Hebrew University, Jerusalem, Israel**
Evolution of gene regulation and dynamics of adaptation

17:20-17:45 Coffee / Tea Break

17:45-18:20 **Dr Eran Meshorer, Hebrew University, Jerusalem, Israel**
Chromatin plasticity and the dual capacity of stem cells to either differentiate or maintain pluripotency

18:20-18:45 **Prof Eugene Rosenberg, Tel-Aviv University, Israel**
The inheritance of genetic information from mothers to offspring by transmission of symbiotic microbiota: the hologenome concept

18:45-19:15 **Discussion** - **Moderators**: Dr Lior David & Dr. Yael Heifetz

20:15 ***Gala Dinner*** (Dan Jerusalem) & Performance

Thursday 2nd June 2011

07:00-08:30 Breakfast

08:30-08:35 **Morning session -** Moderators: Prof Ruth Shalgi & Prof Tom Fleming

08:35-09:10 **Dr Veronique Duranthon**, National Institute of Agronomical Research, Jouy en Josas, France
Dietary effects on gene expression in rabbit embryos and subsequent placental and fetal development

09:10-09:45 **Prof Regine Steegers-Theunissen**, Erasmus University Medical Centre, Rotterdam, The Netherlands
Folic acid and the Periconceptional Developmental Programming of reproductive performance

09:45-10:20 **Prof Jo Leroy**, University of Antwerp, Belgium
Impact of maternal obesity on bovine oocyte competence and embryo developmental potential

10:20–10:50 Coffee / Tea break

Short communications - Moderators: Prof Kevin Sinclair & Dr Veronique Duranthon

10:50-11:10 **Dr Maria Arias-Alvarez, University Complutense of Madrid, Spain**
Effect of acute food deprivation on preimplantation embryo development and quality in rabbit model

11:10-11:30 **Ms Monika Laczmarek, IARFR PAS Olsztyn, Poland**
Early malnutrition: programming of fertility by maternal diet

11:30-11:50 **Dr Hanna Stinshoff, University of Veterinary Medicine, Hannover, Germany**
Dietary CLA supplementation affects luteal mRNA-expression

11:50-12:10 **Dr Zvi Roth, Hebrew University, Israel**
Cellular and molecular mechanisms underlying environmental stress disruption of bovine oocyte developmental competence

12:10-12:30 **Dr Amir Orian, Technion, Israel**
A fly view on sumo-targeted ubiquitin ligase in early development

12:30-13:00 **Discussion** - Moderators: Prof Ruth Shalgi, Prof Tom Fleming, Prof Kevin Sinclair & Dr Veronique Duranthon

13:00-14:00 Lunch

14:00-14:05 **Afternoon session** – Moderators: Prof Nava Dekel & Dr Yael Heifetz

14:05-14.40 **Dr Uzi Moallem,** Institute of Animal Sciences, Volcani Center, Bet-Dagan, Israel
Dietary effects on bovine oocytes and embryos

14:40-15:15 **Prof Ruth Shalgi, Tel-Aviv University, Israel**
Regulation of mouse egg activation and fertilization

15:15-15:50 **Prof Nava Dekel,** Weizmann Institute of Science, Rehovot, Israel
Consequences of endometrial inflammation and injury

15:50–16:10 Coffee / Tea break

16:10-16:45 **Dr Ariel Revel,** Hadassah University Hospital Ein Karem, Jerusalem, Israel
Human oocyte and ovarian tissue: how to preserve fertility

16:45-17:20 **Dr Deborah Kidron,** Meir Medical Centre, Kfar Saba, Tel Aviv University, Israel
Fetal Growth Restriction: an autopsy Series

17:20-17:50 **Final Discussion:**
Periconceptional developmental programming, what is the future?
Open to all participants

18:00 Dinner - Jerusalem

Friday 3rd June 2011

One day Excursion to Masada, Ein-Gedi and the Dead Sea

ORAL PRESENTATION ABSTRACTS

Tuesday 31st May 2011

16:25-17:25

Professor Howard Cedar

Hebrew University, Jerusalem, Israel

Programming DNA methylation during development

DNA methylation derived from the gametes is probably erased during early development and a bimodal pattern of methylation is then re-established at about the time of implantation. While this basic profile is maintained throughout development, targeted demethylation and de novo methylation events during the formation of specific cell lineages. The orchestration of this process takes place according to well-defined rules and is probably directed by sequence information within the DNA itself. Using genetic and epigenetic manipulations in tissue culture and transgenic mice, we have attempted to decipher the mechanisms involved in setting up methylation patterns at different stages of development, thus revealing the factors required for specificity as well as the enzymatic machinery for carrying out the reactions. Our studies also shed light on methylation changes that occur during somatic-cell reprogramming.

Tuesday 31st May 2011

17:25-18:15

Professor Tom Fleming

University of Southampton, UK

Protein undernutrition during the mouse periconceptional period alters embryogenesis, placentation and disease risk into adulthood

The periconceptional period is one where mother and embryo experience several molecular interactions which control both implantation and nutrient supply by the mother and the pattern of development and growth by the embryo. This dialogue can have profound, long-term consequences affecting health into the next generation. In diverse models where such periconceptional interactions are modified by, for example, maternal diet, embryo in vitro culture, or maternal immune reaction, adult disease can be the final result. Evidence for periconceptional developmental programming (PDP) and its association with adult disease has been identified in different mammalian species including the human. For mechanistic understanding, we have focused on a maternal protein undernutrition model in rodents during preimplantation development which leads to changes in fetal and perinatal growth that associate later in life with increased cardiovascular, metabolic and behavioural dysfunction. Here, poor diet changes the amino acid composition of the maternal reproductive tract which induces altered nutrient signalling within the early embryo and its capacity for biosynthesis and growth. The functioning of extra-embryonic lineages of the developing conceptus, responsible for yolk sac and placenta formation, are particularly sensitive to such environmental conditions. Epigenetic mechanisms also contribute to altered developmental programming mediated through maternal diet. Collectively, it is important to develop preventative strategies to protect against the adverse inter-generational effects of PDP. Funded by BBSRC, MRC, NICHD (USA).

Wednesday 1st June 2011

08:35-09:10

Dr Kevin Sinclair

School of Biosciences, University of Nottingham, UK

One-carbon metabolism and epigenetic programming in the oocyte and pre-implantation embryo

Long-term programming effects of specific dietary B-vitamins (e.g. vitamin B_{12}, folate) and sulphur amino acids (e.g. methionine) on key epigenetic processes during gametogenesis and pre-implantation development have been the focus of research endeavours at Nottingham in recent years. In the sheep we have demonstrated that physiologically relevant reductions in the dietary supply of these one-carbon related metabolites to intending mothers can lead to epigenetic modifications to DNA methylation in progeny associated with hypertensive adult offspring that are also insulin resistant; 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 [Maloney et al., 2011. *J. Nutr.* **141:** 95-100]. These studies set a precedent for the long-term programming effects of specific micronutrients during gametogenesis and pre-implantation development. Current studies are seeking to develop our understanding of how disturbances to one-carbon metabolism can lead to epigenetic dysregulation of DNA methylation in germ cells. To that end we have been developing mathematical models of these cycles in the ovary, which have served to direct current in vitro experiments with oocytes and somatic cells from the ovarian follicle. Long-term follow-up studies in sheep offspring also point to endoplasmic reticulum stress as a putative mechanism underlying insulin resistance, and the epigenetic regulation of selected genes involved in this process is currently under investigation. These emerging data together with current concepts and thoughts on future directions will be discussed at the meeting.

Wednesday 1st June 2011

09:10-09:45

Professor Nick Macklon

University of Southampton, UK

Maternal – embryo interactions regulating success of human ART and fertility

Successful implantation and pregnancy are widely viewed as being dependent upon an intimate dialogue, mediated by locally secreted factors between a developmentally competent embryo and a receptive endometrium. Reproductive success in humans is however limited, largely because of the high prevalence of chromosomally abnormal preimplantation embryos.

The transient period of endometrial receptivity in humans coincides with differentiation of endometrial stromal cells (ESCs) into highly specialized decidual cells. The role of cyclic decidualization of the endometrium in the implantation process and the nature of the decidual cytokines and growth factors that mediate the crosstalk with the human embryo are unknown, partly due to the fact that human implantation sites are for ethical reasons inaccessible *in vivo*. We have therefore employed a human co-culture model, consisting of decidualizing ESCs and single hatched blastocysts, to identify the soluble factors involved in implantation and to correlate these to embryo development. Surprisingly, the presence of a healthy developing embryo was to shown to have no significant effect on decidual secretions. In contrast, arresting embryos trigger a strong response, characterized in our studies by selective inhibition of IL-1β, -6, -10, -17, -18, eotaxin, and HB-EGF secretion. When co-cultures are repeated with *undifferentiated* ESCs, none of the secreted cytokines were affected by the presence of a developing or arresting embryo. It appears therefore that human ESCs become biosensors of embryo quality upon differentiation into decidual cells. In view of the high incidence of gross chromosomal errors in human preimplantation embryos, cyclic decidualization followed by menstrual shedding may represent a mechanism of natural embryo selection that limits maternal investment in developmentally impaired pregnancies.

Defective decidualisation may allow suboptimal embryos to implant, which if not ultimately viable, could lead to clinical pregnancy loss. These findings suggest that the endometrium may have a more important role in determining the success of implantation and the health of the conceptus than previously recognized.

Wednesday 1st June 2011

09:45-10:20

Professor Bernd Fischer

Martin Luther University, Germany

Periconceptional developmental programming: Adverse effects of maternal diabetes mellitus on embryo metabolism

Women with diabetes mellitus are subfertile. About 15 % of pregnancies end in abortions/still births. If diabetes is not well treated women face an increased incidence of congenital malformations in their babies. The questions to be answered are “how” and “when” diabetes affects pregnancy. Regarding “when”, we have investigated preimplantation embryos from diabetic and normoglycamic mothers in an experimental rabbit diabetes type I model. Maternal diabetes does reach the relevant developmental compartment directly adjacent to the preimplantation embryo: uterine secretions. In uterine secretions from diabetic rabbits the concentrations of nutrients (glucose, specific amino acids) and growth factors (IGF1, 2) are increased while insulin is decreased. Regarding both questions (“how”, “when”), we found that insulin is essential for blastocyst development in vitro in the rabbit. Developmental progress of blastocysts in vivo, determined by well-defined gastrulation stages and by marker gene analyses, shows (i) a clearly retarded development under diabetic developmental conditions (ii) as early as during the preimplantation period. Relevant receptors of the insulin/IGF family are downregulated. Indications of a disturbed blastocyst metabolism are decreases in glucose transporter 4 (RNA and protein level) and in the expression of key metabolic enzymes like hexokinase, phosphoenolpyruvate carboxykinase and glycogen synthase. Our data have yielded metabolic targets for periconceptional developmental programming which need to be studied now in later developmental stages and with a focus on epigenetic modifications.

Assessing altered uterine conditions and developmental retardation on the one hand and pregnancy rates in diabetic women on the other raises the question whether and if yes, which compensatory mechanisms may be turned on at early ontogenetic stages. We have laid our scientific focus on two vital metabolic pathways: adipokines and adipokine function and CREB signalling in blastocysts developing under diabetic conditions. In the lecture and in accompanying posters our recent findings will be presented.

* funded by DFG (grant NA 418/4-2) and the Wilhelm Roux Programme of the MLU Faculty of Medicine

Wednesday 1st June 2011

10:50-11:10

Dr Francesca Mossa

University College Dublin, Ireland

SW Walsh[1], DA Kenny[2], P Lonergan[1], GW Smith[3], JJ Ireland[3], ACO Evans[1]

[1]School of Agriculture Food Science and Veterinary Medicine, and the Conway Institute, University College Dublin, Ireland; [2]Teagasc Grange Animal & Grassland Research and Innovation Centre Ireland; [3]Department of Animal Science, Michigan State University, East Lansing, Michigan, USA.

Impact of undernutrition on circulating testosterone and cortisol concentrations during pregnancy of cattle

We have shown that undernutrition of heifers during the first trimester of pregnancy diminished the number of follicles growing during follicular waves (antral follicle count, AFC) in female offspring. In pregnant sheep, nutrient restriction in the period of maximal placental growth results in a decrease in maternal cortisol and prenatal testosterone excess enhances fetal early follicular recruitment

We hypothesized that maternal nutritional restriction for the first third of gestation causes an increase in maternal cortisol and testosterone concentrations, which in turn result in a reduction in AFC in offspring.

Beef heifers were randomly assigned to one of two nutritional treatments: Control (C; n=25) or Restricted (R; n=35) and were individually fed at 1.2 or 0.6 of their maintenance (M) energy requirements, respectively, starting 11 days before insemination. Estrous cycles were synchronized and heifers were artificially inseminated with sex-sorted (female) semen from a single sire. From Day 110 to calving all animals received a 1.4 M diet. Blood samples were collected monthly during gestation.

In maternal circulation (pregnant heifers), testosterone concentrations were similar between groups before conception, were lower ($P<0.05$) in the C vs R group during the first 110 days of gestation, and were not different between groups thereafter. Peripheral cortisol concentrations in maternal circulation did not differ between groups at any stage ($P>0.05$). The peak, minimum and mean AFC were lower ($P<0.05$) in single female calves born to heifers in the R (n=10) compared with the C (n=13) group at each age.

In conclusion, maternal nutritional restriction during the first trimester of pregnancy increased maternal testosterone concentrations. Increased testosterone during pregnancy may have a negative impact on follicular development in offspring.

Wednesday 1st June 2011

11:10-11:30

Dr Cristina Ovilo

INIA, Spain

Cristina Ovilo, Antonio Gonzalez-Bulnes, Rita Benítez, Yolanda Núñez, Pilar Pallares, Maria Luz Perez-Solana, Raul Sanchez-Sanchez, Laura Torres-Rovira

Placental gene expression and relationships with maternal reproductive features in an obese porcine breed

The Iberian porcine breed is characterized by an extreme adipogenic trend and low reproductive efficiency. The pattern fits with obesity due to *leptin resistance*, as these pigs are extremely voracious, have increased levels of serum leptin and show genetic polymorphism in the leptin receptor (*LEPR*) related with a lower *LEPR* gene expression. There is scarce information about the factors influencing placental function and pregnancy success in obese conditions. The objective of this work was the evaluation of the relationship of placental gene expression with several maternal reproductive features, narrowly related with the conceptus environment and development, in a pig model for obesity. Seventeen Iberian gilts were sacrificed at day 42 of pregnancy and their genital tracts were collected for morphometric examination and sampling. Also sow endocrine and metabolic status was assessed. Three key genes with known roles on placentation and angiogenesis were selected for mRNA quantification in placental tissue: Leptin (*LEP*), Estrogen receptor α (*ER1*) and Vascular Endothelial Growth Factor A (*VEGFA*). There was a positive linear relationship between the expression of *LEP* and *VEGFA* (r=0.78, P<0.01), and the expression of both genes was also positively correlated with progesterone levels. VEGFA ligand-receptor system is a specific stimulator of angiogenesis and it has been previously shown to be regulated by leptin, in agreement with our findings. The angiogenic effects of both factors at placental level could be the mechanism responsible for the positive effect of progesterone on pregnancy success. Also *VEGFA* gene expression positively correlated with placental efficiency (r=0.74, P<0.05), and thereafter with fetal growth. Despite the limited number of analyzed animals, the joint results suggest a coordinated and positive effect of progesterone, leptin and vascular endothelial growth factor on placental vascularization and thus, on fetal nutrition and development.

Wednesday 1st June 2011

11:30-11:50

Professor Dariusz Skarzynski

Institute of Animal Reproduction and Food Research of PAS, Olsztyn, Poland

Anna Z. Szóstek[1], Marta Botelho[2], Agnieszka Kolomycka[1], Wieslaw Wiczkowski[1], Antonio M. Galvao[2], Mariusz Piskula[1] Graca M. Ferreira-Dias[2]

Can phytoestrogenes disrupt endocrine regulations in the equine uterus?

Phytoestrogenes are naturally occurring estrogens in plants, found in fodder used to feed domestic animals. The presence of phenolic rings in phytoestrogens structure allows for its binding to estrogen receptors (ER). Thus, in this way, phytoestrogens may compete with 17-β estrogen (E2) to influence several endocrine mechanisms during the estrous cycle. The aim of the study was to determine whether phytoestrogens can influence endometrium function in mares by modulation of prostaglandin (PTG) secretion. High concentrations of coumestrol were found in alfalfa. When alfalfa was present in fodder fed to mares, high concentration of coumestrol was found in plasma. In *in vitro* experiments, the effect of coumestrol on the secretion of PTG from equine endometrial epithelial and stromal cells was examined. The cells were obtained from uterus at 2-5 day of estrous cycle. In a preliminary study, the most effective dose of coumestrol and time of action were selected. Thus, the dose of 10^{-8}M of coumestrol and stimulation time of 24h were chosen for next experiments. Mare epithelial (n=4) and stromal (n=4) cells were stimulated with coumestrol (10^{-8}M), E2 (10^{-9}M) and oxytocin (OT, 10^{-7}M; a positive control) for 24h. Coumestrol stimulated PGE_2 secretion from both epithelial cells ($P<0.001$) and stromal cells ($P<0.001$) in comparison to control group. Additionally, coumestrol was also capable of stimulating $PGF_{2\square}$ secretion from epithelial ($P<0.001$) and stromal cells ($P<0.001$). Besides, when E2 treatment was compared to control group, it stimulated PGE_2 secretion from epithelial cells ($P<0.05$) and $PGF_{2\alpha}$ secretion from stromal cells ($P<0.05$). Moreover, the impact of coumestrol and E2 on the proliferative potential of both types of equine endometrial cells was studied. Coumestrol increased epithelial cells viability ($P<0.01$) when compared to control group. These findings suggest that coumestrol can modulate PTG secretion from endometrial cells and may be potentially associated to reproduction tract disorders in mares.

Wednesday 1st June 2011

11:50-12:10

Mr John Twight

Erasmus Medical Centre Rotterdam, The Netherlands

M.E.C. Bolhuis, E.A.P. Steegers, F. Hammiche, W.G. van Inzen, J.S.E. Laven, R.P.M. Steegers-Theunissen

The preconception diet is associated with the chance of ongoing pregnancy in women undergoing IVF/ICSI treatment

Objective

Subfertility is an increasing problem in Western countries. Relative undernutrition, due to a misbalance of macro- and micronutrient intake, affects fertility parameters in both women and men. In this study, we investigate the association between adherence to dietary recommendations in couples undergoing IVF/ICSI treatment and chance of ongoing pregnancy.

Materials and methods

Between October 2007 and December 2010, couples planning pregnancy, visiting the outpatient clinic of the department of Obstetrics and Gynaecology of the Erasmus University Medical Centre Rotterdam, were offered preconception counselling. Self-administered questionnaires on general characteristics and diet were filled out and checked during the visit. Six questions, based on dietary recommendations of the Dutch Nutrition Center, covered the intake of main food groups (bread, fats, vegetables, fruit, meat and fish). From the answers on these questions, we calculated the Preconception Dietary Risk Score (PDR), which provides an overall estimate of personal nutritional habits. Dietary quality decreases with an increasing PDR score. We define ongoing pregnancy as an intra uterine pregnancy, confirmed by echo. We analyzed 172 couples receiving a first IVF/ICSI treatment within a six-month timeframe after preconception counselling. We applied univariate and logistic regression analysis on the outcomes of interest using SPSS.

Results

After adjusting for age of woman, PDR of partner, BMI and couple smoking status, an inverse association was shown between the PDR of the woman and the chance of ongoing pregnancy after IVF/ICSI treatment (β=-0.39, 95% CI=-0.75 – -0.03, p=0.03). Intuitively, a one-point decline on the PDR score, results in a 1.5 fold increased chance of ongoing pregnancy, i.e., OR 1.47 (95%CI=1.03 – 2.12).

Conclusions

Our results show that a higher adherence to Dutch dietary recommendations in women undergoing IVF/ICSI treatment increases the probability of ongoing pregnancy. This data warrants further confirmation in couples achieving a spontaneous pregnancy and in randomised controlled trials.

Wednesday 1st June 2011

12:10-12:30

Dr Susanne Ulbrich

Technical University of Munich, Germany

AE Groebner, V Zakhartchenko, S Bauersachs, I Rubio-Aliaga, H Daniel, HD Reichenbach, HHD Meyer, E Wolf, SE Ulbrich

Do placental abnormalities and fetal overgrowth manifest prior to implantation through imbalances in intrauterine nutrient supply with amino acids?

An increased supply of amino acids (AA) in the uterine lumen prior to implantation is of specific importance for the developing embryo in ruminants[1]. A defective nutrient supply of the preattachment conceptus might contribute to placental abnormalities and fetal oversize often occurring in bovine pregnancies following somatic cell nuclear transfer (SCNT). Therefore we analyzed whether the bovine intrauterine AA concentration is altered in the presence of an *in vitro* fertilized (IVF) vs. SCNT conceptus. Oocytes were fertilized for the generation of IVF-embryos while SCNT embryos were produced from fibroblast cultures. Embryos were cultured under identical conditions to the blastocyst stage until transfer to recipient heifers at day 7. At day 18 post estrus, animals were slaughtered and the uterus was flushed for the analysis of 41 intrauterine AA and derivatives by highly sensitive liquid chromatography-tandem mass spectrometry. Although both IVF and SCNT-produced trophoblasts released similar amounts of interferon-τ (IFN-τ), the intrauterine concentrations of most AA were lower in the presence of SCNT-embryos. While the cationic AA L-lysine, L-arginine and L-histidine were significantly 7.1-fold, 7.0-fold and 5.7-fold higher in the presence of an IVF embryo compared to the non-pregnant control group, AA were only 3.7-fold, 2.8-fold and 4.6-fold higher in SCNT pregnancies ($p<0.05$). Aromatic AA L-phenylalanine, L-tyrosine, and L-tryptophan were 9.2-fold, 7.5-fold and 4.9-fold increased in IVF pregnancies, but only 5.9-fold, 6.1-fold and 4.1-fold higher in SCNT pregnancies with respect to controls. The concentrations of the branched chain AA L-leucine, L-isoleucine and L-valine were significantly 5.3-fold, 7.1-fold and 6.3 higher in the IVF, but only 3.0-fold, 3.7-fold and 3.8-fold higher in the SCNT group when compared to the cyclic controls. Thus, SCNT embryos failed to induce adequate AA transport independent of IFN-τ, wherefore further mechanisms regulating nutrient supply may be hypothesized. Alterations of an optimal supply with AA may imply metabolic challenges affecting the development at later stages of pregnancy and adult life.

[1]*Groebner et al., Increase of essential amino acids in the bovine uterine lumen during preimplantation development. Reproduction, 2011, in press*

Wednesday 1st June 2011

15:35-16:10

Professor Nir Ohad

Tel-Aviv University, Israel

Epigenetic regulation of plant reproduction and development

The plant life cycle alternates between a diploid sporophytic to a haploid gametophytic phases. The gametophyte size and phase dominance were dramatically reduced during angiosperm evolution and have specialized in flowering plants to support the reproductive process. Employing a genetic approach we have identified key regulatory genes which govern phase transition in plants**.** These genes belong to the Polycomb group complex (PcG) which catalyzes histone methylation, facilitating epigenetic control of gene expression profiles. Using *Physcomitrella patens* as a model system to study the function of the PcG genes in early terrestrial plants we find that FIE PcG protein (PpFIE) accumulates in haploid meristematic cells and cells which undergo fate transition during dedifferentiation programs in the gametophyte. In the absence of PcG PpFIE or PpCLF (a PcG SET domain protein), apical stem cells over-proliferate and are unable to develop leafy gametophytes and reach the reproductive phase. However, both PcG mutants develop sporophytic-like structures expressing gene markers unique to this phase. This aberrant phenotype may result from failure of the PcG complex to repress proliferation and differentiation of apical stem cells, designated to become sporophytes. The PpFIE aberrant mutant phenotype can be partially rescued by the *Arabidopsis thaliana* FIE gene, representative of angiosperms, which diverged 450 million years ago from bryophytes. Reciprocally, PpFIE can partially complement the *A. thaliana fie* mutant, thus illustrating functional conservation of the protein throughout plant evolution. Our results indicate that the PcG machinery was harnessed at the onset of the evolution of alternating generations to regulate differentiation of meristematic cells. The role of PcG in controlling the transition between the gametophytic to sporophytic programs in both bryophytes and angiosperms will be discussed**.**

Reference

A. Mosquna, A. Katz, E. L. Decker, S. A Rensing, R. Reski and N. Ohad (2009).Regulation of stem cell maintenance by the Polycomb protein FIE has been conserved during land plant evolution. Development Vol. 136(14) (pp. 2433-2444**)**

Wednesday 1st June 2011

16:10-16:45

Dr Yoav Soen

Weizmann Institute of Science, Rehovot, Israel

Shay Stern[1], Yael Fridmann-Sirkis[1], Erez Braun[2]

[1]Weizmann Institute of Science, Rehovot, Israel; [2]Dept. of Physics & Network biology Research Laboratories, Technion- Israel Institute of Technology, Haifa, Israel

Plasticity of gene regulation and the diversification of developmental programs

Organisms often respond to adverse environmental conditions by invoking pre-selected stress responses. While this strategy is efficient for accommodating known environmental challenges, it may not be adequate for coping with novel challenges against which the organism have not yet acquired a dedicated response program. We investigate the ability of an organism to cope with these conditions by confronting the development of the fly, *D. melanogaster*, with a severe synthetic challenge that could not have likely been encountered throughout its evolutionary history. We show that under a wide range of implementations this challenge can lead to de-canalization of development, which coincides, with acquisition of tolerance to the otherwise lethal challenge. The de-canalization response was mediated by suppression of Polycomb group genes, thus leading to activation of developmental regulators in new domains. Remarkably, some of the developmental modifications were epigenetically inherited by subsequent generations of offspring that did not experience any challenge. These results show that novel environments can induce modified developmental programs that are stable across generations without necessarily being selected in evolution.

Wednesday 1st June 2011

16:45-17:20

Dr Lior David

Hebrew University, Rehovot, Israel

David Lior[1], Ben-Harush Yossef[1], Stolovicki Elad[2], and Braun Erez[2]

[1]Dept. of Animal Sci., Hebrew University of Jerusalem, Rehovot, Israel; [2]Dept. of Physics & Network biology Research Laboratories, Technion- Israel Institute of Technology, Haifa, Israel

Evolution of gene regulation and dynamics of adaptation

The capacity of cells to accommodate frustrating conditions contributes much to their evolvability especially in the face of novel challenges. Harnessing a gene to a new regulatory context (genome rewiring) contributed much to evolution of new functions in organisms. Genome rewiring, however, creates incompatibilities inside cells and little is known on the dynamic process of adaptation that is necessary for establishment of a new regulatory mode. Moreover, genome rewiring might require adaptation potential beyond existing genetic variability, especially in the face of an unforeseen challenge and thus entail adaptation mechanisms other than selection of an advantageous existing mutant. We have rewired ,in yeast, the essential *HIS3* gene to the Gal system by replacing its native promoter with p*GAL1*. In glucose, repression of *HIS3* severely challenges the rewired cells but we have shown that 50% of them adapted to grow competitively on histidine-lacking glucose plates and their growth capacity was inherited. Using chemostat experiments, the dynamic adaptation process of the whole population and of individual cells that were plated from consecutive time points were followed. We found that there were no cells with an advantageous phenotype, neither prior to the glucose exposure nor during the first generations of growth in this environment, indicating that this adaptation relied on individual cells that switched into an adapted state in response to the glucose exposure. We identified mutations associated with growth of rewired cells on glucose but also cases in which probable epigenetic changes contributed to this adaptive phenotype that nevertheless, was stably inherited. Thus, at least on the short term, adaptation is a dynamic and multifaceted process involving genetic and epigenetic factors that can result in multiple solutions to a novel challenge of gene regulation. Our experimental evolutionary approach demonstrated the evolvability potential of yeast cells and the plasticity of gene regulation that relies both on genetic and epigenetic factors.

Wednesday 1st June 2011

17:45-18:20

Dr Eran Meshorer

Hebrew University, Jerusalem, Israel

Chromatin plasticity and the dual capacity of stem cells to either differentiate or maintain pluripotency

Embryonic stem cells (ESCs) are characterized by unique epigenetic features including decondensed chromatin, hyperdynamic association of proteins with chromatin and a permissive transcriptional program. We investigated the mechanisms that regulate chromatin plasticity in ESCs. Using epigenetic drugs and mutant ESCs lacking various chromatin binding proteins, we find that histone acetylation, G9a-mediated histone H3 lysine 9 (H3K9) methylation and lamin A expression, all control chromatin protein dynamics in ESCs. Histone acetylation controls, almost exclusively, euchromatin protein dynamics, lamin A expression regulates heterochromatin protein dynamics, and G9a-mediated effects are both euchromatic and heterochromatic. Altered chromatin dynamics was associated with perturbed ESC differentiation. Expression microarrays and ChIP-seq experiments for H3K9ac before and after HDAC inhibition highlighted genes involved in extra cellular matrix as involved in pluripotency. Together, these data delineate the mechanisms responsible for chromatin plasticity in ESCs, and indicate that the epigenetic state of the genome modulates chromatin plasticity and the differentiation potential of ESCs.

Wednesday 1st June 2011

18:20-18:45

Professor Eugene Rosenberg

Tel-Aviv University, Israel

The inheritance of genetic information from mothers to offspring by transmission of symbiotic microbiota: the hologenome concept

The hologenome theory of evolution considers the holobiont (the animal or plant with all of its associated microorganisms) as a unit of selection in evolution. The hologenome is defined as the sum of the genetic information of the host and its microbiota. The theory is based on four generalizations, each of which is supported by a large body of empirical data: (1) All animals and plants establish symbiotic relationships with microorganisms; often the genetic information of the diverse microbiota exceeds that of the host. (2) Cooperation between the host and the microbiota contributes to the fitness of the holobiont. (3) Variation in the hologenome can be brought about by changes in either the host or the microbiota genomes; under environmental stress, the symbiotic microbial community can change rapidly by a variety of mechanisms including microbial amplification, horizontal gene transfer and acquisition of new microorganisms from the environment. (4) Symbiotic microorganisms are transmitted between generations. These points taken together suggest that the genetic wealth of diverse microbial symbionts can play an important role in development, adaptation and evolution of higher organisms. The distinguishing feature of the hologenome theory is that it considers all of the diverse microbiota associated with the animal or the plant as part of the evolving holobiont. The hologenome concept can be considered epigenetic and Lamarckian (within a Darwinian framework) because offspring can inherit acquired characteristics without changes in the host genome.

Thursday 2nd June 2011

08:35-09:10

Dr Veronique Duranthon

National Institute of Agronomical Research, Jouy en Josas, France

Dietary effects on gene expression in rabbit embryos and subsequent placental and fetal development

Administration of a hyperlipidemic hypercholesterolemic (HH) diet to rabbit does both during the pre and post conceptional periods has been shown to induce Intra Uterine Growth Retardation (IUGR) and reduce birth weight. In contrast, IUGR did not occur when dams were fed only from fertilization onwards. These results pointed out to the periconceptional period as a key period for the induction of IUGR in response to the HH diet. First, the analysis of endocrine responses to breeding indicated that puberty was advanced in HH fed females. Secondly, the histological structure of ovaries collected from these HH fed females was characterized. Despite a similar number of primordial follicles, the total number of antral follicles was reduced and this was associated with an increased number of atretic follicles. We thus wondered whether oocytes from these ovaries were able to initiate normal embryonic development after fertilization by control males. The embryonic transcriptome of HH and control F1 embryos was analyzed and compared at the key period of embryonic genome activation (EGA : 8-16 cell stage in the rabbit). This stage of development was selected because it results directly from the reprogramming activity of the oocyte. ADIPOPHILIN transcript was found transiently overexpressed at EGA. This overexpression is subsequently followed by an overexpression of the adipophilin protein at the blastocyst stage, where it co-localises with an increased number of lipid droplets in the cytoplasm of embryonic cells. Very interestingly this increased lipid content is still detectable in the trophoblast of F1 conceptuses at Day 28 of gestation. Gene expression analysis of lipid and cholesterol metabolism in these placentas indicated that the expression of LDL-receptor, CD36 and LXR-alpha was significantly decreased in the HH group. In order to study transgenerational effects, F1 females were fed either the HH or the control diet, thus resulting in 4 experimental groups according to the diet of the individual and of its mother (i.e., C-C, C-HH, HH-C and HH-HH). Placentae from pregnant F1 females (F2 placentae) were collected at D28 of pregnancy. The lipid content of the placentae was shown to depend both on F0 and F1 female diets. Ultrastructural analyses showed that a large number of lipid droplets could be found in the trophoblast layer of C-HH, HH-C and HH-HH placentas compared to the C-C placentas. Moreover, dilated smooth endoplasmic reticulum was observed only in HH-C placentas and multilamellar lipoid lysosomes were identified only in the trophoblast layer of HH-HH placentae.

Taken altogether, these data establish that a maternal high fat diet can affect puberty onset, ovarian function, gene expression at early embryonic stages and placental structure in the first and second generation offspring.

Thursday 2nd June 2011

09:10-09:45

Professor Regine Steegers-Theunissen

Erasmus University Medical Centre, Rotterdam, The Netherlands

Folic acid and the Periconceptional Developmental Programming of reproductive performance

The periconception window is one of the most important periods in life, covering a period of at least 10 weeks before conception up to 8 weeks after conception. During the preconception period the maturation of the gametes takes place and the endometrial tissues are being prepared for pregnancy. The first 2 months of pregnancy are essential for implantation, embryogenesis and placentation. Insults during the periconception period can result in fertility problems and the development of congenital malformations and placental related adverse outcomes, e.g., fetal growth restriction, with consequences for health and reproductive performance in later life.

The natural B-vitamin folate plays a key role in human reproduction, especially during the periconception period. A shortage of folate during this period is associated with an increased risk of congenital malformations, such as neural tube defects, congenital heart disease, and fetal growth restriction. Moreover, the periconception use of synthetic folic acid in a supplement or a folate-rich dietary pattern reduces the risk of having a child with a neural tube defect or severe growth restriction.

Last decade, evidence is accumulating that the preconception folate status is also related to fertility due to its influence on oocyte quality and sperm count. First studies in human and animals show that the methyl-groups provided by folate and/or methionine attenuate the ovarian response to gonadotrophin stimulation during in vitro fertilization cycles. This suggests that folate also affects follicular metabolism. The methyl-groups are used for DNA methylation, which is one of the best studied epigenetic mechanisms so far. The modification of DNA-methylation may be one of the underlying mechanisms in which folate exerts its effect on human reproduction.

In this presentation the importance of folate and folic acid during the periconception window in the developmental programming of reproductive performance will be addressed.

Thursday 2nd June 2011

09:45-10:20

Professor Jo Leroy

University of Antwerp, Belgium

Impact of maternal obesity on bovine oocyte competence and embryo developmental potential

Maternal metabolic disorders are characterized by a rise in non-esterified fatty acid (NEFA) concentrations in the blood due to up regulated lipolysis. In addition, overconsumption of energy dense diets also alters the plasma lipid content. Those metabolic disturbances have been linked to reduced fertility. More specifically, there is growing evidence that a decrease in oocyte and preimplantation embryo quality may partly account for this subfertility syndrome. In our research group, we hypothesized that a deviating maternal metabolism, associated with altered lipid content in the blood, is reflected in the micro-environment of the maturing oocyte and developing embryo, possibly affecting fertility through hampered oocyte developmental competence and embryonic quality, metabolism and viability.

In a bovine model, we collected follicular fluid (FF) and demonstrated that several metabolic changes in the blood result in concomitant changes in the FF of the dominant follicle. Similar correlations have been found in women, indicating that obese women have a deviant FF composition. Oocytes aspirated from these women displayed a lower chance to become high quality embryos. To study the pathogenesis of the latter observations, we mimicked elevated NEFA concentrations during *in vitro* oocyte maturation. Oocytes were less competent to develop to embryos after NEFA-exposure and the cumulus cell investment displayed increased rates of apoptosis. The embryos that did reach the blastocyst stage showed a deviant energy metabolism, oxygen consumption and amino acid turnover. Furthermore, some major key genes related to embryo energy metabolism and quality were differently expressed and mitochondrial activity was also affected. Finally, hyperlipidemia due to overfeeding may directly affect the preimplantation embryo quality. In conclusion, the maturing oocyte and preimplantation embryo are vulnerable for metabolic disorders which may partly explain the subfertility seen in these patients.

This work has been supported by FWO-Vlaanderen and BOF-UA.

Thursday 2nd June 2011

10:50-11:10

Dr Maria Arias-Alvarez

University Complutense of Madrid, Spain

Pablo Bermejo-Alvarez, Rosa Maria Garcia-Garcia, Pilar G Rebollar, Osama Sakr, Gabriele Brecchia, Alfonso Gutierrez-Adan, Cristiano Boiti, Pedro L Lorenzo

Effect of acute food deprivation on preimplantation embryo development and quality in rabbit model

Food deprivation suppresses ovulation and affects female fertility. The aim of this work was to study the effect of short-term fasting before fertilization on several parameters after artificial insemination (AI). Rabbits does were fed *ad-libitum* (AL group, n=6) or fasted (FA group, n=6) during 72h prior to AI and thereafter refed with standard diet during 84h. In that time point serum insulin, glucose, and progesterone (P_4) concentrations were measured. Ovulation rate (OR) and embryo recovery rate were recorded after laparotomy. Embryos were classified by morphological criteria and blastocyst quality was analyzed by gene expression. mRNA transcripts was quantified by qRT-PCR to contrast relative levels of histone *H2az* and: tumor protein p53 (*TP53)*, insulin-like growth factor 2 receptor (*IGF2R)* and solute carrier family 2 (facilitated glucose transporter) member 4 (*SLC2A4*). Statistical analysis was performed by χ^2 and t-student test. OR (13.4±1.7 *vs.* 12.8±2.3) and serum P_4 concentrations (1.5±0.4 *vs.* 1.4±0.4ng/ml) were not significantly affected. Fasted does showed lower serum glucose (112.0±18.6 *vs.* 245.0±46.8mg/dl, P<0.001) and higher insulin concentrations (41.3±5.97 *vs.* 16.7±5.9µUI/ml, P<0.02) than does fed *ad-libitum*. The number of embryos recovered/female was significantly lower in FA than in AL group (9.0±1.4 *vs.* 4.4±1.6, P<0.05). The number of morulae was similar between groups (1.4±1.1 *vs.* 2.0±0.8), but in AL group most of the embryos were blastocysts compared to fasted rabbits (5.4±1.5 *vs.* 1.6±0.6, P<0.05). However, the expression patterns of the quality-genes studied did not differ. In conclusion, acute fasting before AI alters metabolic status and seems to affect embryo development and kinetics; OR, corpora lutea function and blastocyst quality genes studied were not affected.

We acknowledge MEC for funding.

Thursday 2nd June 2011

11:10-11:30

Ms Monika Kaczmarek

IARFR PAS Olsztyn, Poland

Mendoza Tamra[2], Kozak Leslie P.[1,2]

[1]Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland;
[2]Pennington Biomedical Research Center, Baton Rouge, LA, USA

Early malnutrition: programming of fertility by maternal diet

Several epidemiological studies have shown that malnutrition during fetal and neonatal development causes changes in the body's structure, metabolism and hormonal sensitivity in a way that affects the ability of the individual to adapt to its environment in later stages of life. The mechanisms by which transient perturbations in the nutrient environment of the infant generate long-term consequences on disease susceptibility are very poorly understood. One evolutionary adaptation allowing an individual or species to survive when food is scarce is hypothalamic hypogonadism. This phenotype, especially important to female reproduction, is representative of a negative energy balance and is reversible when energy supplies are restored. Hypothalamic hypogonadism is also common phenotype in obesity and is caused by deficiencies in leptin signaling through deregulation of hypothalamic pathways. It is seems more likely that perturbations that occur in circulating levels of leptin and other hormones, including hormones of the reproductive axis, known modulators of brain development, will certainly be perturbed by early malnutrition. To date, several attempts have been made to establish that a developmental window, sensitive to nutritional programming, also exists in regard to reproduction and fertility. Our studies with mice show that, under-nutrition during lactation, accompanied by severe hypoleptinemia, suppresses gene expression of the LEPR/KISS1/GPR54/GnRH pathway in the hypothalamus of both males and females in association with attenuated gonadal development. However, most importantly, a delayed puberty was also observed in first and second generation offspring, even though they had never been exposed to malnutrition. Global gene expression analysis showed altered gene expression in the hypothalamus of first generation females. In conclusion, the nutritional environment changes selective patterns of gene expression in the hypothalamus and gonadal development of first generation females and can affect the future reproductive performance and hormonal status of both daughters/sons and granddaughters/grandsons raised in an apparently healthy environment.

Thursday 2nd June 2011

11:30-11:50

Dr Hanna Stinshoff

University of Veterinary Medicine, Hannover, Germany

Eike Onnen-Lübben, Sandra Wilkening, Ana Hanstedt, Heinrich Bollwein, Christine Wrenzycki

Dietary CLA supplementation affects luteal mRNA-expression

The peripartal phase of dairy cows is dominated by a negative energy balance. This - among other effects- has a negative influence on the reproductive performance of these animals. Conjugated linolic acids (CLA) may present an opportunity to weaken this negative influence by optimizing the energy balance post parturition.

Forty healthy Holstein Frisian cows and heifers were randomly allocated to two treatment groups (Group 1: 10 cows, 4 heifers; group II: 7 cows, 4 heifers) and one control group (10 cows, 5 heifers). Animals of group I were fed 50g of a CLA/day/animal, animals of group II orally received 100g CLA/day/animal and the animals of the control group received no CLA.

Animals of all groups were subjected to a standard synchronisation protocol followed by aritificial insemination (AI). In each group 4 animals conceived. A transvaginal corpus luteum biopsy was performed on days 7, 14, and 21 post AI in all animals. Non-pregnant animals were also biopsied (Group I n=4, group II n= 4, control group n=3). Animals deemed pregnant on d 28 were again biopsied on day 42.

A sensitive RT-qPCR assay was used to analyse the relative abundance of nine gene transcripts (VEGF, bCOX, 3ß-HSD, bECE-1, PPARγ-2, PGHS, PGF2αR, cPLA-2, STAR) all relatated to luteal function.

The following results could be obtained:

1) The expression of 3ßHSD-, bECE-1-, PGF2αR- and STAR-mRNA was affected by the treatment (0g vs. 50g vs.100g CLA), status (pregnant vs. non-pregnant) and day of cycle or pregnancy, respectively.
2) The treatment showed an effect on the relative abundance of PGHS.
3) The expression of VEGF was mainly affected by the day of cycle/pregnancy.
4) The transcript level of bCOX was influenced by treatment and status of the animals.
5) The expression of PPARγ-2 and cPLA-2 were not affected by treatment, status or day of biopsy.

Acknowledgements: The authors would like to thank the DFG (German Research Foundation) for their financial support (PAK286/1; WR154/1-1).

Thursday 2nd June 2011

11:50-12:10

Dr Zvi Roth

Hebrew University, Israel

Cellular and molecular mechanisms underlying environmental stress disruption of bovine oocyte developmental competence

Mammals' ovarian oocyte pool arrests at the diplotene stage of the first meiotic prophase, when oocytes undergo a growth phase. In view of global climate change and increasing awareness of pollutants as biologically active molecules, the potentially deleterious effects on oocytes of females exposed to such environmental stressors were studied.

Exposing bovine oocytes to physiologically relevant thermal stress induced apoptosis through the sphingomyelin pathway, expressed in a high proportion of oocytes with increased caspase activity and DNA fragmentation. Heat shock also impaired nuclear maturation events, such as cytoskeleton rearrangement and spindle apparatus formation, resulting in less oocytes progressing to metaphase II. Seasonal heat stress delayed the first two embryonic cleavages and reduced the proportion of oocytes that cleaved and developed to the blastocyst stage, concurrent with alterations in the level of mRNA-encoding genes involved in maturation (*C-MOS, GDF9*) and embryonic development (*GAPDH*, *POU5F1*), before and after embryonic genome activation.

Oocyte developmental competence was also impaired by a range of phthalate esters, a class of water-insoluble synthetic organic chemicals that are widely used in industrial applications. Exposure of cumulus oocyte complexes to dibutyl phthalate (DBP), di(2-ethylhexyl)phthalate (DEHP) or its primary metabolite mono(2-ethylhexyl)phthalate (MEHP) during maturation reduced the cleavage rate and the proportion of embryos that developed to the blastocyst stage, with altered patterns of gene expression, notably reduced expression of *POU5F1* and increased expression of *ASAH1*, a pro-apoptotic factor involved in ceramide metabolism.

These emerging data in farm animals must be carefully considered in evaluating potential risks to humans. Exposing the ovarian pool of oocytes to environmental stressors appears to impair maternal mRNA storage and/or mechanism of transcription renewal and to have long-term detrimental consequences on the development of preimplantation embryos.

Thursday 2nd June 2011

12:10-12:30

Dr Amir Orian

Technion, Israel

Mona Abed, Bella Kulton, Eliya Lotan-Bitman Olga Pesin, Jeff Delrow, Susan Parkhurst

A fly view on sumo-targeted ubiquitin ligase in early development

Transcriptional co-factors are essential for embryonic development. Often they serve as molecular 'hubs' connecting signaling to transcription and mediate crosstalk between pathways. Degringolade (Dgrn), is a new cofactor for the Hairy/E(spl) (HES) family of developmental bHLH regulators, encodes a RING finger/E3 ubiquitin ligase. Dgrn and its mammalian ortholog RNF4 are SUMO Targeted Ubiquitin Ligases (STUbLs). STUbL's are the molecular machinery connecting the SUMO pathway with ubiquitylation: they bind to SUMOylated proteins via their SUMO- interaction motif (SIM) domains and facilitate substrate ubiquitylation. *dgrn* null mutants are female sterile, producing embryos that arrest development after 2-3 nuclear divisions. These mutant embryos exhibit fragmented or de-condensed nuclei and accumulate higher levels of SUMO-conjugated proteins, suggesting a role for Dgrn in genome stability. Dgrn displays dynamic subcellular localization, accumulates in the nucleus at times when HES/Hey family members are active, and is required for HES/Hey family activity during sex determination, segmentation and neurogenesis. We find that HES family members, are ubiquitylated by Dgrn, and that Dgrn is also a negative regulator of the HES–related co-repressor Groucho (Gro/TLE). Dgrn inhibits Gro-containing repressor complexes by inactivating Gro via sequestration, and concomitantly SUMO-independent ubiquitylation of the repressor. In both cases Dgrn affects protein-protein interactions and protein localization. Genome-wide DamID chromatin profiling analysis identified direct loci co-bound by Dgrn and Gro, suggesting that Dgrn antagonism to Gro is relevant beyond its interaction with HES repressors. Our results indicate that Dgrn is essential for proper early Drosophila patterning during embryogenesis, and is a novel regulator of the Notch-HES pathway during early fate determination.

Thursday 2nd June 2011

14:05-14:40

Dr Uzi Moallem

Institute of Animal Sciences, Volcani Center, Bet-Dagan, Israel

Dietary effects on bovine oocytes and embryos

The objectives were to determine the incorporation of dietary specific FA into follicular fluid (**FF**), granulosa cells and cumulus-oocyte complexes (**COC**) in dairy cows, and the effects on ovarian follicular development, preovulatory follicles, and the quality of oocytes that were aspirated *in vivo* (**OPU**) and were then *in vitro* maturated and *in vitro* fertilized (**IVF**). In the first experiment, feeding unsaturated FA increased the diameter of the preovulatory follicles and the concentrations of androstenedione and estradiol and estradiol/progesterone ratio in the FF. The mRNA expression of the steroidogenic enzyme Aromatase was higher in the unsaturated FA group. In another experiment, the proportion of C18:3n-3 in plasma was 5.5 times as high in cows fed with omega-3 than in the controls. The proportion of C18:3n-3 in FF was 7 times higher in follicles obtained from cows fed omega-3 than that in other groups and that of C20:4n-6 was lower in follicles of the omega-3 group. The proportion of C18:3n-3 in granulosa cells was fivefold in the omega-3 cows compared with the control, and in COC from the omega-3 group it was 4.7%, whereas this FA was not detected in the controls. Follicles from omega-3 cows had lower progesterone concentrations than those from the controls, and lower E_2 concentrations than those of the omega-6 group. Number of follicles in the ovaries was higher in the omega-3 group than in the control. Oocyte cleavage rates were higher in the omega-3 group than in the control, from which it could be concluded that dietary omega-3 may enhance oocyte quality. We have also observed modifications in the timing of hormonal and behavioral events around estrus in the omega-3 cows. The present study demonstrates that accurate and specific nutrition can influence many aspects of the reproductive system, among them some that could improve fertility performance.

Thursday 2nd June 2011

14:40-15:15

Professor Ruth Shalgi

Tel-Aviv University, Israel

Regulation of mouse egg activation and fertilization

Meiosis of mammalian oocytes commences during embryonic life and arrests around birth, at prophase of the first meiotic division, characterized by the presence of a germinal vesicle. Resumption of meiosis is initiated by germinal vesicle breakdown, followed by completion of the first meiotic division, as manifested by extrusion of first polar body (PBI), and by an arrest at metaphase of the second meiotic division (MII). The fertilizing spermatozoon triggers a series of events that take place within the fertilized oocyte and are collectively referred to as “oocyte activation”. The two early events of oocyte activation are cortical granules exocytosis that establishes the block to polyspermy and the release from the MII arrest and continuation of meiosis. Extrusion of the second polar body indicates completion of meiotic division.

Src family kinases (SFKs) are non-receptor protein tyrosine kinases that are expressed in many cell types, including oocytes and play an important role in many developmental processes. Fyn, an SFKs member, takes part, among other processes, in cell adhesion, organization of cytoskeleton, myelination, lymphokine secretion and more. Recent studies, including ours, imply a role for Fyn in meiosis and mitosis. Other studies demonstrate that SFKs, particularly Fyn, are required for regulation of microtubules polymerization and spindle stabilization.

Using murine oocytes as a model, we imply a role for Fyn in several signaling events key processes that regulate the exit from metaphase in oocytes and zygotes. Our results elucidate the cellular and molecular processes that lie at the base of possible errors occurring during these fundamental developmental processes.

Thursday 2nd June 2011

15:15-15:50

Professor Nava Dekel

Weizmann Institute of Science, Rehovot, Israel

Gnainsky Y[1], Granot I[2], Barash A[2], Or Y[2], Levin D[2], Dekel N[1]

[1]*Department of Biological Regulation, The Weizmann Institute of Science*
[2]*IVF Unit, Department of Obstetric, and Gynecology, Kaplan Medical Center, Rehovot, Israel*

Consequences of endometrial inflammation and injury

Acquisition of uterine receptivity, an essential prelude for successful embryo implantation, is fully dependent on the development of adequate conditions for the attachment of the conceptus to the endometrial epithelium. The particular constituents of such “adequate conditions” are not as yet defined and markers for a receptive endometrium are practically unavailable. Furthermore, the disappointing, poor rate of pregnancy, presently achieved following the transfer of high quality embryos makes implantation the rate-limiting step for the success of *in vitro* fertilization. A substantial increase in pregnancy rate, induced by endometrial biopsy in patients with recurrent implantation failure, has been reported by us and confirmed by others. Along this line, we have later demonstrated that uterine dendritic cells are crucial for implantation in mice. Taking these findings into account we raised the hypothesis that local injury generated by endometrial biopsy increases uterine receptivity by provoking inflammation**.** The overall goal of our study was to unveil the role of inflammation in successful implantation, further providing valuable clinical information that will be translated into diagnosis and treatment of infertility. Our experiments were specifically directed at 1. Characterization of the response of human endometrial cells to inflammatory-inducing agents. 2. Examination of the effect of immune cells on endometrial cell differentiation and 3. Establishment of biomarkers for predicting implantation competence. The results of our study suggest a mechanism by which endometrial biopsy treatment increases the success of pregnancy, unveiling the role of inflammatory cytokines and specific immune cells in acquisition of endometrial receptivity. Most importantly, our research identifies a potential biomarker for implantation competence. This information will potentially define new clinical strategies to treat infertility when implantation fails.

Thursday 2nd June 2011

16:10-16:45

Dr Ariel Revel

Hadassah University Hospital Ein Karem, Jerusalem, Israel

Human oocyte and ovarian tissue: how to preserve fertility

Preservation of fertility potential has become an important part of treatment of young oncologic patients that face risk of infertility due to cancer therapy. Reproductive organs are highly sensitive to the cytotoxic effect of chemotherapeutic drugs and radiotherapy that causes premature gonadal insufficiency and leads to hormonal dysfunction and gamete cell loss. Dialogue between the oncologist and the fertility specialist is essential in order to provide the optimal counselling and care for the patient.

Hadassah Medical Center offers its patients various options for fertility preservation. Decision as to the suitable preservation procedure is based on patient's gender, age, type of disease and subsequent treatment regimen, fertility and family status as well as preference. Advanced methods for gonadal tissue cryopreservation are being developed in Hadassah. Recently, a healthy baby boy was delivered to a survivor of bone marrow transplantation due to Thalasemia major, who had her ovarian cortex cryopreserved and subsequently reimplanted.
Further investigation and improvement of fertility preservation techniques brings new hope and genuine prospect of post-treatment parenthood for young cancer survivors.

Thursday 2nd June 2011

16:45-17:20

Dr Deborah Kidron

Meir Medical Centre, Kfar Saba, Tel Aviv University, Israel

Julia Eidel, Reuven Sharony

Fetal Growth Restriction: An autopsy Series

Fetal growth restriction (FGR) is associated with increased mortality and morbidity, both both perinatal and long term. In cases of intrauterine demise or termination of pregnancy, fetal autopsy can be carried out. The fetal autopsy includes detailed gross and histological evaluation of fetus and placenta. It encompasses multiple features: external features (including dysmorphism), estimation of growth (by weight of internal organs), signs of active disease (i.e. inflammation, edema, thrombosis), malformations of internal organs and abnormalities of placenta.

In 15 years (1996-2010), 894 autopsies of second and third trimester fetuses were performed with complete reports at Meir Hospital, Kfar-Saba, Israel. Among these, 270 (30%) were small for gestational age SGA), based on weight and measurements. Terminations of pregnancy (TOP) were carried out in 142 cases whereas 128 fetuses died in utero. Indications for TOP were malformations, chromosomal aberrations, oligohydramnios and FGR in various combinations.

A variety of qualitative and quantitative observations in autopsy reflect the effect of deprivation of oxygen and nutrients on the organs and tissue of the developing fetus. The changes involve multiple systems, including cardiovascular, hematopoietic, coagulation, intestinal, renal, endocrine, skeletal and nervous. The study of accompanying placentas adds information relating to etiology of FGR.

Fetal autopsies shed light on the severely affected end of the spectrum of changes in FGR. They offer insight into the function and interrelationship of developing organs and tissues under suboptimal conditions.

POSTER PRESENTATIONS FROM ABSTRACTS

POSTER PRESENTATION INDEX

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Poster 1

Danielle Bebbre (Presenting Author Sergio Ledda)
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Custom microarray analysis of gene expression in oocytes derived from adult and prepubertal sheep during in vitro maturation

Aim of this work was to evaluate changes in the transcriptome (mRNA levels) during in vitro maturation of oocytes deriving from adult and prepubertal ovine donors using a customized ovine cDNA microarray.

In collaboration with Porto Conte Ricerche S.r.l. and Illumina, Inc., we generated a medium density microarray based on Illumina DASL technology; it comprises 1536 independent assays addressing 1039 transcripts relevant to several cell functions such as transcription, cell cycle regulation, signal transduction and immune response. Sequences were selected in the OAGI (Ovis Aries Gene Index) database of the Dana-Farber Cancer Institute Gene Index Gene Ontology (GO) section (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=sheep).

Immature (GV) and in vitro matured (IVM) oocytes deriving from adult and prepubertal ovine donors (3 groups of 20 oocytes for each class) were subjected to mRNA isolation with Dynal microbeads kit (Oslo, Norway) and subsequently hybridized to the customized microarray.
Comparison of mRNA content between adult and prepubertal GV oocytes revealed 14 differentially expressed genes, mainly upregulated in prepubertal gametes, while 20 genes were differentially expressed at the MII stage (13 upregulated in adults and 7 in prepubertal oocytes), ($P<0.01$).

After in vitro maturation, 44 genes showed different abundance between the GV and the MII stage of adult-derived oocytes (41 transcripts more abundant in GV and 9 in MII stage), while 31 mRNA showed different levels in prepubertal gametes (19 transcripts more abundant in GV and 12 in MII), ($P<0.01$). Notably, only one transcript was seen to vary during meiotic progression of both adult and prepubertal gametes. Quantitative reverse transcription Real Time PCR validated the expression profile of six selected transcripts.
This study confirms differences in the transcriptome of GV and IVM oocytes deriving from adult or prepubertal sheep. The differences observed during meiotic progression suggest that prepubertal and adult oocytes may have different needs to undergo meiosis.

Poster 2

Charlotte Dupont
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INRA, France

Role of leptin in milk for developmental programming using a rabbit model

Leptin is a cytokine produced mainly by adipose tissue, which regulates energy consumption through hypothalamic regulation of food intake. The postnatal leptin surge, described particularly in rodents, has been demonstrated to be crucial for hypothalamic maturation and brain development, but also the maturation of numerous organs, among which pancreas. Leptin deficiency during the neonatal period increases the incidence of type 2 diabetes, obesity and metabolic disorders, together with hyperphagia in offspring, due to deficient hypothalamic maturation. Injections of exogenous leptin in the neonatal period reverse these adverse effects.

Milk is known to contain leptin and it was shown that breastfeeding has a protective effect against obesity. Moreover, p*er os* supplementation of rat neonates with physiological doses of leptin during the suckling period provides a protection against excess weight gain and obesity in adulthood, improves insulin sensitivity and changes food preferences. The aim of this study was to establish the role of leptin in milk in developmental programming using a rabbit model.

Forty-eight newborn female rabbits were treated orally just before suckling (once a day) with either leptin (L), leptin antagonist (LA) or saline (S) for 10 days. After weaning, they were fed with one of two diets: control (C) or "Obesogenic diet" (Ob), supplemented with lipids and sugar.

At puberty, LA females fed with the Ob diet became significantly heavier and tended to be fatter than controls. However, the protective effect of leptin was not confirmed in the L group fed the Ob diet, although intestinal absorption of leptin was confirmed in preliminary experiments. Treatments did not affect glycemia, nor plasma leptin and insulin concentrations.

The results indicate that the specific blockage of the absorption of leptin in milk during the neonatal period may lead to metabolic consequences in offspring fed excess fat and sugar.

Poster 3

Anita Franczak

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Genomic profile of pregnant and cyclic porcine endometrium – our concept of microarray data analysis

Gene expression microarrays have already strong position in the field of reproductive physiology research. The greatest advantage of microarrays and, at the same time, the biggest disadvantage of this technique is the abundance of obtained data. It is hard to extract the relevant data from huge amount of information about the expression of hundreds or thousands genes. The fold-enrichment calculations based on gene ontologies or creation of integration networks of genes will not provide clear output until performed in the context of the results obtained using other methods, e.g. in situ hybridization, PCR or immunohistochemistry. The aim of our study was to analyze the data obtained from microarrays based on our previously achieved novel, but still preliminary results. Previously, we found that during the period of embryo-maternal cross-talk porcine endometrium produces steroid hormones and expresses steroidogenic enzymes. To search for the regulations and networks of uterine steroidogenesis we compared the genomic profile of gravid (n=4) and cyclic (n=4) porcine endometria harvested on days 15 to 16 of pregnancy and the estrous cycle. We used Two-Color Agilent's Porcine Gene Expression Microarray. We found that from total amount of genes in porcine genome, 2378 genes were differentially expressed when compared pregnant and cyclic endometrium. The expression of 1424 genes from 2378 genes was changed more than two-fold ($p<0.05$). We found that from 19 genes generally involved in steroidogenesis, 11 genes were differentially expressed in pregnant and cyclic porcine endometrium. Thus, this approach allows to create more accurate hypothesis to exam the role of uterine steroidogenesis in embryo-maternal interaction. We also can use this concept to analyze other interesting phenomena, such as prostaglandins pathways or immune responses in the uterus during periconceptional development.

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Poster 4

Rosa García-García
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Influence of energy supplementation during gestation and lactation on the viability of offspring and their ovarian development in the rabbit model

The aim was to assess the growth rate and viability of a total of 101 litter of rabbit NZ females supplemented with 2% of propylene glycol in water during mid-gestation to weaning at 25 days (group PG; n=17) or during lactation period to weaning (group PL; n=42). The rest of animals were allocated in control group (group C; n=42). Mean body weight of litters and mortality rate were assessed from born to weaning and from weaning to 8-weeks. The ovarian follicular apoptosis in 14-weeks offspring females were studied by TUNEL assay. Body weight of offspring of PL group at 21 days were significantly higher than C group (2.30 ± 0.04 *vs.* 2.11 ± 0.04 Kg; P <0.05) and similar to PG group (2.16 ± 0.07 Kg). Mean mortality rate during lactation period didn't vary among groups (7.8 %). However, body weight from weaning to 56 days decreased for PG progeny (8.67 ± 0.51 *vs.* 10.4 ± 0.32 and 10.6 ± 0.31 Kg for PL and C group, respectively) as well as mortality rate (25.6 ± 5.12 *vs.* 12.7 ± 3.6 and 8.4 ± 1.96%, respectively). In fact, PG group showed 1 young rabbit less than the mean of the other two groups at 8 weeks (5.4 *vs.* 6.4 young rabbits; P< 0.05). Healthy follicles rate was similar between groups (28.4 ± 12.5 *vs.* 27.08 ± 14.1 *vs.* 27.5 ± 7.5% for C, PG and PL group) as apoptotic follicle index (49.8 ± 11.0 *vs.* 43.5 ± 10.9 *vs.* 52.9 ± 8.0, respectively) but early atretic follicles rate (<50% of apoptotic cells) were slightly higher for PG group than PL group (29.4 ± 8.8 *vs.*19.5 ± 3.5, P<0.09). Studies on embryo quality are under way. In conclusion, energy supplementation with propylene glycol during gestation period affected viability of progeny whereas no effect was shown with the addition in lactation time.

Poster 5

Antonio Gonzalez-Bulnes

Laura Torres-Rovira, Pilar Pallares, Susana Astiz, Maria Luz Perez-Solana, Pedro Gonzalez-Añover, Raul Sanchez-Sanchez and Antonio Gonzalez-Bulnes

INIA, Spain

Fat content in the diet affects metabolic parameters of early conceptuses of an obese swine breed

Obesity and nutritional habits are associated with appearance of early miscarriages and alterations of embryo/foetal growth. Previous studies performed on Iberian sows, a breed with a high potential for obesity due to a polymorphism in the leptin receptor gene (*LEPR*) related with a lower *LEPR* expression, indicate an increased weight of early embryos from dams with life-time high-fat feeding. Current study aimed to evaluate metabolic features of amniotic and allantoic fluids of Iberian early-conceptuses which may be related to differences in embryo growth. Data were obtained from 14 sows that were fed either with a standard diet having 2.8% of fat (group C, n=7) or with a fat-enriched diet (6.3%, group HF) from 18 weeks-old. At 47 weeks-old, the animals become pregnant and at Day 25 of pregnancy, entire genital tracts were collected and amniotic and allantoic fluids were obtained by aspiration through the amniochorion and chorioallantoic membranes, respectively, for evaluation of parameters related to metabolism of lipids (triglycerides and cholesterol). Results indicate that HF sows, those animals with heavier embryos (1.2 ± 0.0 vs 0.9 ±0.1 g for C; $P<0.0005$), showed a significant lower level of cholesterol in allantoids (3.4 ±0.2 mg/dl vs 6.3 ± 0.3 for C; $P<0.05$) and triglycerides both in allantoids and amnions of conceptuses (17.4 ± 6.0 vs 37.7 ± 3.4 mg/dl and 17.9 ± 4.3 vs 41.6 ± 11.2 mg/dl; $P<0.001$ for both). Cholesterol is of vital importance as key constituent of cell membranes and precursor of hormones and metabolic regulators whilst triglycerides are largely known as a main source of energy for the developing conceptuses. Thus, alterations in growth patterns of embryos from mothers with increased fat content in the diet suggest an adaptive response similar to the previously described in rodents with altered maternal nutrition.

Poster 6

Julia Knelangen

Alexander Navarrete Santos[1], Bernd Fischer, Anne Navarrete Santos

Martin Luther University, Germany

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The influence of glucose on epigenetic modifications during adipogenic differentiation of mouse embryonic stem cells

The increasing incidence of obesity is a worldwide problem. Epidemiological studies evidenced that programming of obesity occurs as early as during embryonic and fetal development. The molecular mechanisms of metabolic programming of embryonic cells still remain unclear. We hypothesized that microRNA (miRs) play an important role in adipogenic cell differentiation and pathogenesis of obesity and are involved in metabolic programming.

We used the pluripotent murine embryonic stem cell (ESC) line CGR8 to investigate the role of miRs during adipogenic differentiation. An early, transient treatment of the CGR8 cells with retinoic acid, followed by culture with insulin and triiodthyronine, gives rise to functional adipocytes within 21d (Dani et al. 1996). The employed model encompasses the full process of adipogenesis and provides hereby a suitable model to particularly study the initial stages of adipocyte determination and differentiation.

We have characterized the dynamic expression profile of miRs during adipocyte maturation in vitro by microarrays containing the Exiqon v.8.1 probe set which targets nearly 1500 miR sequences from mouse, human and up to 50 other species (Knelangen et al. 2011).

To study potential mechanisms of metabolic programming, we induced a metabolic stress by culturing the ESC during determination phase in media with high or low glucose (0mM-100mM). Long-term effects induced by glucose on adipocyte differentiation were analyzed by FACS. It allowed us to separate mature adipocytes from progenitors and undifferentiated cells and to determine the differentiation efficiency. A short-time exposure to low glucose decreased the amount of finally adipogenic cells by about 43 %, demonstrating a direct influence of glucose on the adipogenic differentiation efficiency of the CGR8 cells. Our data indicates that glucose availability determines the adipogenic programme in embryonic cells. In summary miRs are involved in embryonic and mesenchymal cell differentiation into adipocytes. Glucose affects adipogenesis as soon as during ESC determination.

Poster 7

Emma Lucas
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Altered DNA Methyltransferase Expression in Preimplantation Mouse Embryos is induced by Maternal Low Protein Diet

Epigenetic changes during early development are an attractive candidate mechanism for establishing the long-term effects of sub-optimal maternal diet in offspring. We have reported previously that expression of mRNAs for the DNA Methyltransferase enzymes (Dnmts), which are responsible for the establishment and maintenance of methylation patterns, is altered in several offspring tissues in mouse, including E17.5 placenta and in adult kidney and heart, in a tissue and sex-specific manner, following administration of maternal low protein diet during gestation.

Dams were either fed a normal protein diet (18% casein; NPD), a low protein diet (9% casein; LPD) or a high protein diet (30% casein; HPD) throughout the preimplantation period. Blastocysts were collected at E3.5 and *Dnmt* mRNA expression was examined in single blastocysts by RT-qPCR.

Relative expression of maintenance methyltransferase *Dnmt1* mRNA was found to be 1.35-fold upregulated ($P<0.05$) in male blastocysts from dams fed LPD, versus blastocysts from NPD and HPD-fed dams, although no effect of the diet was observed in expression of the *de novo* methyltransferases *Dnmt3a* and *Dnmt3b*, or the methyltransferase-like transcript *Dnmt3L*. Dnmt1 is thought to function in preimplantation development to maintain essential methylation at specific genomic loci, including imprinted genes which are associated with growth. Immunolabelling for Dnmt1 and Dnmt3b in blastocysts shows cytoplasmic and nuclear localisation, respectively. Analysis of Dnmt protein expression intensity across treatment groups is currently underway.

Levels and organisation of DNA methylation are remodelled post-fertilisation to generate embryonic chromatin appropriate for development. Methylation is re-established in blastocysts as inner cell mass and trophectoderm lineages form. Disruption of normal methylation patterns by the blastocyst stage may have long-term implications for development. We are now investigating whether this increase in Dnmt1 expression is associated with altered methylation within the blastocyst.

Poster 8

Sara Maffei
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Molecular mechanisms involved in the response of pig ovaries to seasonal heath stress

Decreased fertility during the hot season is a common problem in pigs. Maternal hyperthermia reduces oocyte fertilizability and increases embryonic mortality. Cell biochemical thermoprotection mechanisms involve members of the heat shock protein (Hsp) family. Hsp40, also known as Mammalian Relative of DnaJ (MRJ) protein, plays a pivotal role as co-chaperone after heat shocks.

Although this protein may be involved in local thermoprotection, no information is available on the role of Hsp40 in mammalian ovary.

Hsp40 in pig, was characterized by extracting RNA from ovaries, granulosa cells and from pools of 5 oocytes. The nucleotide sequence showed an homology of 95% with the human, 92% with the bovine and 84% with the mouse orthologs.

Western blot analysis with anti-Hsp40 antibody, identified an immune peptide, displaying a MW 38Kda, in agreement with results obtained in other species.

Immunofluorescence studies, showed that Hsp40 is found in oocytes, granulosa and theca cells of follicle at all developmental stages.

Exposure of porcine ovaries to 42°C for 1 hour resulted in a significant increase ($P<0.05$) of Hsp40 mRNA levels (2,4 ± 0,35 fold) in oocytes, while no significant raise was detected in cumulus cells.

Furthermore, pig ovaries were collected at the slaughterhouse during the cold and hot season and semi-quantitative analysis was performed. A comparison of transcript levels, revealed higher expression of Hsp40 in the hot versus cold season.

To our knowledge, this is the first demonstration that Hsp40 is expressed and responds to a thermal stress in pig ovary. Since this co-chaperone acts upstream to other heat shock protein –such as Hsp70- and it is specifically up regulated in the oocytes, our findings suggest that it may play an important protective role against heat stress infertility.

Poster 9

Maria Schindler

Sünje Fischer, René Thieme, Bernd Fischer and Anne Navarrete Santos

Martin Luther University, Germany

CREB-mediated embryo-maternal crosstalk in rabbit blastocysts

Prior to implantation embryo development is regulated by maternal and embryonic factors, interacting at the cellular level without placental barrier. The production and release of maternal factors like insulin, IGFs and glucose play a major role in maintaining cell viability and survival of the embryo. Insulin and IGFs regulate metabolic and mitogenic effects during early embryo development.

CREB is a downstream component of the insulin/IGF cascade. Transcriptional activation requires phosphorylation of CREB and binding as a dimer to a conserved CRE element. The hormone adiponectin is an endogenous factor produced by the mammalian blastocyst and is known to be regulated through activation of CREB. Adiponectin is essential for energy homeostasis and acts synergistically with insulin, in respect to glucose uptake.

We hypothesised that CREB signalling is a determining pathway in the embryo-maternal crosstalk, regulating embryonic gene expression in response to maternal factors. Using the rabbit blastocysts as model of an early preimplantation embryo we could demonstrate that CREB is a functional and regulatory transcription factor of embryoblast cells. CREB phosphorylation was increased by insulin and IGF1 in in vitro cultured blastocysts and the expression of the CREB target adiponectin was reduced.

In diabetic, hypoinsulinaemic rabbits CREB partly compensates for insulin loss and glucose excess. Blastocysts from diabetic mothers showed a significantly reduced CREB phosphorylation and an increased adiponectin expression, compensating for the lack of maternal insulin to maintain the embryonic glucose uptake. Confirmatively to our hypothesis the stimulatory function of adiponectin on embryonic glucose uptake was shown in vitro in blastocysts from healthy rabbits as well as from diabetic mothers.

We demonstrated that CREB participates in maternal and embryonic interactions, connecting the maternal insulin signalling with embryonic metabolism. Within this interaction the CREB-regulated embryonic adiponectin expression is one connecting link between changes in maternal insulin supply and embryonic metabolic adaptation.

Poster 10

Gabriela Siawrys
University of Warmia and Mazury in Olsztyn, Poland

Effect of LH and steroids on leptin secretion by luteal cells during early pregnancy and serum leptin levels during early pregnancy and the oestrous cycle in pig

Leptin, the product of the obesity gene, is implicated in the control of food intake (inhibition) and several reproductive functions. It is postulated to be a regulator of puberty onset, fertility, implantation, maintenance of pregnancy and also embryo growth and development. At the ovary level, leptin might participate in the ovulation process, formation of corpus luteum, angiogenesis and ovarian steroidogenesis. Recently, leptin gene and protein expressions have been localised in the porcine corpus luteum during early gestation. Moreover, it was reported that LH and steroids take part in the regulation of leptin mRNA expression in the porcine luteal cells during early pregnancy.

The aim of these studies was: 1] to examine the effects of LH (1; 10; 100 ng/ml), E_2 (0.02, 0.2; 2; 20 ng/ml) and P_4 (20; 100; 200 ng/ml) on leptin release by porcine luteal cells on days 30-32 of gestation; 2] to compare the levels of serum leptin between pregnancy (days 14-16 and 30-32) and luteal phase of the oestrous cycle (days 10-12 and 14-16). Isolated luteal cells after preliminary culture (48 h) were treated for 24 h with LH, E_2 and P_4. The concentrations of leptin in the media samples and serum were measured using a leptin RIA kit.

It was shown that:1] E_2 (0.02 ng/ml) and P_4 (100 ng/ml) significantly increased leptin secretion by porcine luteal cells on days 30-32 of gestation; 2] there were no significant differences in leptin serum level between early pregnancy and luteal phase of the oestrous cycle. The present findings indicate that steroids are involved in the regulation of leptin secretion by porcine luteal cells during early pregnancy and suggest that leptin can affect function of luteal cells during this period.

This research was supported by the Ministry of Science and Higher Education (Project: N N311 098634)

Poster 11

Hardy Manser
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A retrospective study into the association of maternal periconceptional negative energy balance on progeny reproductive performance: the dairy cow paradigm

Developmental programming is likely to have profound implications for the efficiency of livestock production. Given the decreasing reproductive performance (fertility being the top reason for low survivability), with selection for high milk yield and the homogenous nature of the genetics of Holsteins, it is thought that an environmental factor (maternal metabolic stress at conception) may be involved. During early lactation dairy cows usually experience Negative Energy Balance (NEB), especially in pasture based systems where nutritional intake can be limited or of lessened quality. This paper will present the influence of the dam's energy balance at conception on offspring reproductive success. It is hypothesised that animals that are inseminated during periods of NEB will produce offspring who will have decreased reproductive performance.

Of the Holstein heifers recorded in this pasture fed data set, Dams of offspring were stratified for NEB using production variables including Days in Milk (0-90 or >90), Total and Peak Yield, Milk Protein at conception and Fat to Protein Ratio at mating. The relationship between estimated Energy Balance and Offspring reproductive performance (30 day In Calf Rate (ICR), 60 day ICR and Percentage Not Pregnant at mating end) was studied.

Preliminary evidence demonstrates that NEB has a significant association with offspring performance.

This study highlights the need for tightly controlled studies where dam's Energy Balance is accurately measured.

Further studies are required to establish the precise mechanisms of action by which dam's periconceptional metabolic stress affect progeny reproductive performance and specifically the duration and the intensity of NEB at mating on foetal programming of the reproductive axis in Holsteins.

Poster 12

Anne Tarrade (Presenting Author: Pascale Chavatte-Palmer)
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INRA, France

Impact of a maternal hyperlipidic hypercholesterolemic diet on placental function, in a rabbit model

OBJECTIVES: A hyperlipidic hypercholesterolemic diet in prepubertal rabbits has been shown to restrict fetal growth and increase offspring susceptibility to obesity. To better understand this IUGR phenotype, placental function has been explored.

METHODS: Female rabbits were fed with a control diet (C) or a high fat diet (HF) (6% of soybean oil and 0.2% cholesterol) from 10 weeks of age, throughout pregnancy. At D28 of gestation, they were anesthetized and a laparotomy was performed. Blood was drawn from fetuses for total cholesterol and triglycerides measurements. Placentas were collected for transmission electron microscopy and for gene expression including lipid and cholesterol metabolism using q-PCR.

RESULTS: At 28 days, fetal weight and the fetal/placenta ratio were significantly lower in the HF group compared to the C group. Interestingly, total cholesterol concentrations, in fetuses, were not statistically different in C and HF, whereas triglyceride concentrations were increased significantly in HF fetuses. The histological analysis of placentas revealed an abnormal accumulation of light vesicles localized in the trophoblast layer of the HF group, which were subsequently shown by ultrastructural analysis to be lipid droplets. Transcriptomic analysis of lipids and cholesterol metabolism indicated that the maternal HF diet lead to a significantly decrease in the expression of LDL-receptor, CD36 and LXR-alpha in placenta, while genes such as PPARgamma, FATP-4, adipophiline, HMG-coA reductase, SREBP-2 were not affected.

CONCLUSION: Maternal HF diet induced a dyslipidemia in IUGR fetuses, associated with numerous lipid droplets and a modulation of genes implicated in lipid and cholesterol homeostasis in the placenta. It is interesting to note that cholesterol metabolism appears to be preferentially down regulated compared to fatty acids metabolism. Work is ongoing to confirm and validate these data.

Poster 13

John Twigt

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Preconception folic acid supplement use modulates the ovarian response to ovarian stimulation treatment in women

The B vitamin folate is implicated in reproductive outcomes, also after in vitro fertilization (IVF). Folate is a methyl donor, hereby affecting DNA methylation profiles and gene expression profiles. We investigated the effects of folic acid supplementation on ovarian response to mild and conventional forms of stimulation in women.

Materials and Methods

In a randomized controlled trial among subfertile women, 24 subjects received conventional ovarian stimulation and 26 subjects underwent mild ovarian stimulation. Blood samples were obtained for determination of serum estradiol, folate and Anti-Mullerian Hormone before treatment was commenced and after treatment on the day of hCG administration. Folic acid supplementation was determined by questionnaire and validated by serum folate concentrations. Preovulatory follicles were visualised, counted and diameters recorded using transvaginal ultrasound. Linear regression analysis was applied with adjustments for potential confounders.

Results

Estradiol response after conventional ovarian stimulation treatment is modulated by serum folate levels ($\beta^{interaction}$ = 0.52, [0.07-0.97]; p=0.03), this effect was independent of AMH levels and preovulatory follicle count. In the conventional protocol, mean follicle number was greater in non-supplemented women compared to the supplemented group (14.1 vs. 8.9, p=0.03).

Conclusion

Independent of AMH and preovulatory follicle count, folic acid supplementation modulates the estradiol response to conventional ovarian stimulation therapy. Mechanisms of interest for future study are aberrant DNA methylation of ovarian regulatory proteins, modulation of folate-expressed proteins and the production of reactive oxygen species.

Poster 14

Svetlana Uzbekova
Sylvain Auclair, Rustem Uzbekov, Valerie Labas, Christine Perreau, Rozenn Dalbies-Tran

INRA, France

Differences of developmental potential, transcriptome, proteome and ultrastructure between cumulus denuded and cumulus enclosed bovine oocytes after in vitro maturation

Cumulus cells (CC) surround an oocyte and play an important role in acquiring of its competence to be fertilized and to assure early embryo development. CC help oocyte to metabolize energy substrates, stimulate glutathione synthesis and protect from oxidative stress induced apoptosis during in vitro maturation (IVM). The objective was to compare cumulus denuded (CDO) and cumulus enclosed (CEO) oocytes after IVM in metabolically optimized serum free medium. In each experiment, a half of CEO oocytes aspirated from cows' 3-6 mm follicles were denuded and then CDO and CEO were cultured for 22 hours in TCM199 enriched medium. Both groups didn't differ in nuclear maturation rate after IVM; however after in vitro fertilization, the cleavage rate, blastocysts to cleavage ratio and blastocysts' quality were significantly lower in CDO as compared to CEO. In contrast, no such significant differences were found between these groups subjected to ionomycin/6-DAB partenogenic activation.

Transcriptomic analysis was performed using bovine 22K oligo-microarray; 54 known genes mainly involved in regulation of transcrption, binding of RNA and metal ions, metabolism and enzymatic activity were found differently expressed between CDO and CEO. The over-expression of *LEO1* and *COX3* in cumulus-enclosed and *MED10* in CDO was confirmed by real-time RT-PCR. Protein profiling of single oocytes from both groups (n=12) were performed by Intact Cells MALDI-TOF Mass Spectrometry. Among 131 detected m/z species, four peaks showed different intensity between CDO and CEO ($p<0.05$).
Mature CDO and CEO metaphase-II oocytes were analysed by transmission electron microscopy. Significant differences were found in quantity and distribution of lipid droplets, vacuoles and mitochondria between these two groups.

Denuded oocytes before IVM are required for experimental reasons such intracytoplasmic injections in functional knock-down studies. This study brought new insights to understand the role of cumulus cells on oocyte developmental competence after IVM and the oocyte actors involved.

Poster 15

Agnieszka Waclawik
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The expression and regulation of prostaglandin E2 receptor (PTGER2) in the porcine conceptus

Both endometrium and conceptus synthesize and secrete elevated prostaglandin E_2 (PGE_2) into uterine lumen and utero-ovarian circulation before implantation, during the maternal recognition of pregnancy in the pig. We have shown recently that PGE_2 stimulates expression of cyclooxygenase-2, PGE_2 synthase and PGE_2 receptor (PTGER2/ EP2) in the porcine endometrium. Moreover, the endometrial PTGER2 expression is up-regulated on days 11-12 of pregnancy. The objective of the present study was to establish expression pattern of PTGER2 in the conceptus/trophoblast and to determine effect of PGE_2 on the conceptus during periimplantation period. Conceptus/trophoblast samples were collected from pregnant gilts (n=30) during periimplantation period, between days 10 and 25 of pregnancy. Cells isolated from 14-day conceptuses obtained from 6 animals were cultured to 80-90% confluency and incubated for 24 h with vehicle only (control) or PGE_2 (100 nM). PTGER2 expression was examined by Real-time RT-PCR and/or Western blot analysis. Cells isolated from 14-16 day conceptuses were incubated with vehicle only (control) or PGE_2 (100 nM), and then cellular adherence to the ECM fibronectin was measured by CytoMatrix cell adhesion assay. PTGER2 mRNA content was elevated in conceptuses/trophoblasts on days 14-25 of pregnancy (implantation and early placentation period vs. days 10-11, $p<0.05$). Similarly, expression of PTGER2 protein was three-fold greater on days 14-19 than on days 10-11 of pregnancy ($p<0.05$). PGE_2 induced PTGER2 mRNA expression by 70% compared to treatment with vehicle ($p<0.05$). These studies suggest an important role of trophoblast PTGER2 in implantation in the pig. Increased amounts of PGE_2 in uterine lumen during periimplanation period stimulate conceptus PTGER2 expression and acting through this receptor may increase trophoblast adhesion.

Poster 16

Bartosz Wojciechowicz
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The activity 3*β*-hydroxysteroid dehydrogenase/Δ^5-Δ^4 and progesterone release by porcine uterus during early pregnancy

Steroid hormones are produced by porcine endometrium and myometrium during early pregnancy. This period is critical for embryos and requires protection of corpus luteum and production of progesterone (P_4). During early pregnancy the highest embryo mortality occurs. We hypothesized that uterine steroids in pigs may supplement the amount of steroid hormones produced by embryos and corpus luteum and that they are involved in the process of maternal recognition of pregnancy. In this study we examined: 1/ endometrial and myometrial activity of 3*β*-hydroxysteroid dehydrogenase/Δ^5-Δ^4 (3*β*-HSD) – an enzyme converting pregnenolone into P_4 and 2*/ in vitro* production of P_4 by uterine slices harvested from pigs on days 10 to 11, 12 to 13 and 15 to 16 of pregnancy and the estrous cycle. The activity of 3*β*-HSD in the endometrium and the myometrium was measured with histochemistry. Individual endometrial and myometrial slices were pre-incubated *in vitro* for 18 hours and then incubated for the next 12 hours. The concentrations of P_4 in medium were determined with radioimmunoassay. Both endometrial and myometrial tissue expressed of 3*β*-HSD and produced P_4 during early pregnancy and the estrous cycle. Uterine 3*β*-HSD activity was similar during days 10 to 11 and 12 to 13 both in gravid and non-gravid pigs and decreased on days 15 to 16 of pregnancy and the estrous cycle ($P<0.05$). Endometrial P_4 release did not differ among examined days of pregnancy and the estrous cycle ($P>0.05$) but it was higher in pregnant than cyclic pigs on days 12 to 13 and 15 to 16 ($P<0.05$). Myometrial P_4 release was the highest during days 12 to 13 of pregnancy and on days 15 to 16 of the estrous cycle ($P<0.05$). These results provide a novel evidence on the role of the uterus as a source of P_4 during early pregnancy in pigs.

Poster 17
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HP1BP3 is a novel member of the histone H1 multigene family, with essential roles during development

The linker histone H1 binds to the DNA in nucleosomes and plays an important role in establishing and maintaining higher order chromatin structures. There are 11 different subtypes of linker histones in mammals, and they appear to be mostly functionally redundant, as knockout mice of various subtypes appear phenotypically normal. Here we describe a novel and divergent member of the H1 family present in all vertebrates, currently named Heterochromatin Protein 1 Binding Protein 3 (HP1BP3). In higher eukaryotes all H1 variants have the same general structure, consisting of a central conserved globular domain and less conserved N and C terminal tails. HP1BP3 also contains these domains, but it has three conserved globular domains rather than one, and it is twice the size of the other subtypes. Phylogenetic analysis shows that it is most closely related to the intriguing H1foo. Chromatin binding affinity and nuclear dynamics of HP1BP3 are similar to those of other linker histones as assayed by Fluorescence Recovery After Photobleaching (FRAP). Furthermore, as with other subtypes, the C terminal tail is a major determinant of binding affinity, and binding is affected by core histone acetylation. On the other hand, HP1BP3 has additional regions not present in other H1 subtypes, and these also dramatically modulate chromatin binding. A targeted mutation mouse model in which HP1BP3 has been knocked out now reveals that HP1BP3 possesses novel and non-redundant functions. Mice homozygous for the loss-of-function are under-represented in litters, suggesting partial embryonic lethality. The null mice that are born are smaller than their heterozygous and WT littermates, and this reduced body weight is maintained at least until the age of three months. Thus, we have described for the first time a vital, non-redundant function in a novel subtype of linker histone H1.

Poster 18
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Mitochondrial sequestration as a mechanism for restriction of caspase activity during the nonapoptotic process of sperm terminal differentiation in *Drosophila*

Caspases are key executioners of apoptosis, but also participate in a variety of vital cellular processes.Little is known about how cells avoid excessive caspase activity and cell death during nonapoptotic processes. In *Drosophila*, spermatids remove their bulk cytoplasmic contents during terminal differentiation in a process that requires apoptotic proteins, including active caspases. Our recent work suggested that a Cullin-3–based E3 ubiquitin ligase complex is required for caspase activation during this process (also called individualization). This E3 complex, in turn, is regulated by a pseudosubstrate inhibitor protein, dubbed Soti, which is expressed in a subcellular gradient and consequently promotes the formation of a similar gradient of a caspase inhibitor protein. Thus, caspase activation occurs in an inverse graded fashion, protecting the distal spermatid regions, which are the last to individualize, from excessive caspase activity and cell death.

Here, we discovered that active caspases are also expressed in speckle-like structure in the region of individualization. Furthermore, we present evidence that these structures are of mitochondrial origin. Similarly, Klhl10, the substrate recruitment subunit of the Cullin-3-based complex, is also expressed in these speckles, as well as throughout the spermatids. Importantly, we identified a mitochondrial protein of the Krebs cycle, Succinyl-CoA Synthetase (SCS) subunit, which specifically binds to the Cullin-3-based complex. SCS expression dramatically increases throughout the spermatids at the onset of individualization and is also co-localized with Klhl10 within the mitochondrial speckles. Finally, knock down of SCS blocks caspase activation and spermatid individualization, a phenotype identical to *cullin-3* and *klhl10* mutant spermatids, suggesting that SCS is a positive regulator of this E3 complex in spermatids. These findings uncover a novel mechanism of mitochondrial sequestration as a means to restrict caspase activation in a nonapoptotic process.

Poster 19

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The activation of the endometrial TLR 2 affect human trophoblast cells binding to endometrial cells in vitro

Rationale & Hypothesis: The endometrium is the outer lining of the uterus responsible for the reception of the embryo. The main group of pathogen-recognition receptors present in the endometrium is the Toll-like receptors (TLRs) family. Recently, the activation of TLR5 has been found to affect trophoblast cells binding to endometrial cells in vitro. We hypothesize that activation of other members of the TLR family, such as TLR2/6 heterodimer could interfere with the attachment of human trophoblast cells to endometrial cells *in vitro*.

Objectives: To assess the effect of TLR2/6 heterodimer activation on the attachment of human trophoblast cells to endometrial cells *in vitro*.

Methodology: JAr spheroids and hTERT-EECs that mimic trophoblasts and endometrium, respectively, were used in our experiments. hTERT-EECs were co-incubated with 50 JAr spheroids for 0.5, 1, 2 and 4h to estimate the suitable time to assess their attachment efficiency. The best co-incubation time was used to evaluate the effect of TLR2/6 ligand (FSL-1) on JAr attachment. hTERT-EECs were pre-incubated with FSL-1 (0, 1, 10, 100 ng/ml) for 24h. Thereafter, 50 JAr spheroids were added for 1h and the number of attached spheroids evaluated. To study the kinetics of TLR2/6 activation on implantation, hTERT-EECs were either pre-incubated or not with the optimal concentration of FSL-1 (10 ng/ml) for 0.5, 1, 2 and 4h and the attachment of JAr spheroids assessed. Finally, we blocked TLR2/6 in the hTERT-EECs by incubating for 1h with an anti-TLR6 antibody (10ng/ml) and then stimulating the hTERT-EECs with FSL-1 (10 ng/ml) for 24h. JAr spheroids were added for 30min and the attachment was evaluated.

Findings: The attachment of the Jar spheroids increased proportionally with the co-incubation time. Pre-treatment of the hTERT-EECS with TLR2/6 ligand decreased the attachment of the trophoblast spheroids in a concentration-dependent manner. This effect seems to be specific since blocking of the TLR2/6 function restored the JAr spheroid binding to the hTERT-EEC. We conclude that activating TLR2/6 may play a role in human implantation failure.

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