THE OPENING OF BARRIER 3

In June we had a grand opening of our new barrier 3 and we want to share with you the presentations from the scientific programme. Therefore this edition of the newsletter contains more pages than normally.

We hope you will enjoy reading the material and you are welcome to contact us if you want more information. We thank all of you who participated in the opening of the Barrier.
DEAR READER,
Here in Denmark the summer is drawing to a close, and new winds are blowing. Most of our employees have enjoyed their summer holiday, which for all was well deserved as the whole company worked hard during the final stages of the building, cleaning and opening of Barrier 3. The culmination was the official Grand Opening of our new barrier. As CEO of Ellegaard Göttingen Minipigs, I was truly pleased to see so many close business partners and friends here at our facilities in Dalmose, Denmark, in June for our Grand Opening, and I want to express my sincere gratitude to all those who shared the opening with us – both by being here in person or by sending us greetings from around the world – it meant a lot to all of us here at Ellegaard.

At the opening I noted a genuine interest in our entire scientific programme of broad topics concerning the minipig. And I enjoyed the comments and questions inspired by the lectures, as well as the new connections and liaisons, which I know originated on this day! At Ellegaard we believe in collaboration – it is one of our values. We also believe in science, knowledge and know-how and in sharing our expertise, all the while that knowledge will always meet knowledge and that our know-how will help others to make progress. And, as I mentioned, we believe in networking among those who know the Göttingen Minipig! Not only when we are gathering people here in Denmark or through the Minipig Research Forum, but in general. So if you are ever in need of specific knowledge or know-how or just feel that someone might be able to help you with a little helpful advice regarding minipigs, never hesitate to ask us – if we do not know ourselves, we probably know who does!

This leads me to some of the expertise we hold in-house: housing, handling and dosing of minipigs. Of course this is an area of expertise for us, as we work with minipigs on a daily basis and have been doing so for more than twenty years. Also, our know-how is constantly growing based on the feedback we receive from our many customers who are in turn gaining their own valuable experience. In connection with this, it is clear that our customers who have visited our facilities for a handling and dosing course, or who have been visited at their own facility by one of our customer support and training staff have gained a new and improved foundation for working with the minipig. It is clear that this service is of great value, and once again I wish to extend an invitation to you to use our qualified customer support and training or other veterinary services. Please contact us directly at ellegaard@minipigs.dk or check out our website – www.minipigs.com – for full information on these services.

Since our last newsletter, our veterinary services have made a focused effort to gain new scientific data on the Göttingen Minipig. We are pleased to be able to provide new organ weights and blood values – read more about this later in this newsletter.

Autumn is coming and new winds are blowing. We are collecting new knowledge and data, forging new liaisons, friendships and collaborative bonds. We enjoy these positive signs of progress, as we are also convinced our business partners do. At the same time we are pleased to see the things that are running smoothly without changes – such as our solid production of the Göttingen Minipig, which is healthy, steady and ready for your inquiries. And with the completion of Barrier 3, we can meet all the demands for minipigs in the years to come – you can count on that. I hope you enjoy the rest of the summer and a pleasant autumn to all!

Jens Ellegaard, CEO
Ellegaard Göttingen Minipigs A/S was founded on the idea that scientists in biomedical research were in need of a better non-rodent research model with clearer advantages over the established models of dogs and primates. We envisioned a research model that would have many similarities to humans, be small and easy to handle and have a clean and known health status. In other words, we wanted to give the researchers an optimal research model that could support them in their quest for clear results! We believe that our Göttingen Minipig comes close to this vision.

We believe in science, in knowledge, in know-how and in sharing our expertise, all the while that knowledge will always meet knowledge and that our know-how will help others achieve progress. And we believe in networking among those who know the Göttingen Minipig!

Even though we have grown considerably since we started more than 25 years ago, our core values remain the same: we believe in high standards of animal welfare, in showing respect in everything we do, in closely collaborating with one another and our customers, and in complying with high standards of quality for our Göttingen Minipigs, our work and our services. We hope that our values permeate everything we do!

Looking forward, we see a world of possibilities and a lot of uncharted territory yet to be explored. We expect to excel in these efforts, but also realise how the stability of our products and core services is essential for those with whom we work. Therefore you can also expect small, clean, microbiologically defined Göttingen Minipigs, highly reliable deliveries, service and support as well as access to a network of science and knowledge from Ellegaard Göttingen Minipigs in the future. That’s a promise I dare to keep!

As Chief Executive Officer of Ellegaard Göttingen Minipigs, it is my personal goal that you will always experience how we, at Ellegaard Göttingen Minipigs, work hard to make a difference for you. I sincerely hope this is obvious!

Sincerely,

Jens Ellegaard
In the 1980’s, before the construction of the barrier facilities, Lars Ellegaard had a stable with conventional minipigs. Here he is offloading a group of minipigs.

Here are the fields before any of the current barriers were constructed. The first sod was cut in 1992.

In 1993 barrier 1 and the first part of the administration building was constructed and officially opened.

In 1998 barrier 2 was constructed and here is a picture from 2003, when barrier 2 was extended.

Over the years we have attended a lot of exhibitions and meetings to distribute knowledge and information about our unique Göttingen Minipigs. Here is a picture of Lars Ellegaard at an exhibition in Maastricht in the early 1990’s.

In August 2003 we flew a minipig herd to the United States, where the Göttingen Minipigs are bred and distributed by Marshall BioResources.
In 2008 the construction of barrier 3 was started, and June 12th 2009 we officially open the new barrier.

<table>
<thead>
<tr>
<th>Time</th>
<th>Subject</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>08:30-09:30</td>
<td>Registration and pre-tour of the facility</td>
<td>Jens Ellegaard, CEO</td>
</tr>
<tr>
<td>09:30-09:40</td>
<td>Welcome and introduction</td>
<td>Dr. PhD. Aage Kr. Olsen Alstrup, LAB Research, Denmark</td>
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<tr>
<td>09:40-10:10</td>
<td>Minipig history and genetic management</td>
<td>Prof. Dr. Henner Simianer, Göttingen University, Germany</td>
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<tr>
<td>10:10-11:10</td>
<td>Tour around the Barrier 3 / Coffee</td>
<td>Dr. Helmut Ehali, Huntingdon Life Sciences, UK</td>
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<td>11:10-11:35</td>
<td>Immunotoxicity testing in the minipig</td>
<td>Dr. André H. Penninks, TNO, The Netherlands</td>
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<td>11:35-12:00</td>
<td>What you can see in the eye of the Ellegaard Göttingen minipig</td>
<td>Jens Ellegaard, CEO</td>
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<td>12:00-13:00</td>
<td>Lunch</td>
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<td>13:00-13:15</td>
<td>Handling of a minipig (Demonstration)</td>
<td>Adrian Zeltner, Customer Training &amp; Support, Ellegaard Göttingen Minipigs</td>
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<tr>
<td>13:15-13:35</td>
<td>Inhalation Toxicology: New validation</td>
<td>Dr. Wolfgang Koch, Fraunhofer Institute of Toxicology, Germany</td>
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<td>13:35-14:05</td>
<td>Cloned Alzheimer's Model in Göttingen Minipigs</td>
<td>Dr. Arne Lund Jørgensen, Aarhus University, and Ida E. Holm, Randers hospital, Denmark</td>
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<td>14:05-14:25</td>
<td>Convulsions in Minipigs</td>
<td>Christina Skytte, DVM and Henrik Søeborg, DVM LAB Research (Scantox), Denmark</td>
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<td>14:25-14:35</td>
<td>Anaesthesia and analgesia in Ellegaard Göttingen Minipigs</td>
<td>Dr. PhD. Aage Kr. Olsen Alstrup, Aarhus University Hospital, Denmark</td>
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<tr>
<td>14:45-15:00</td>
<td>Closing remarks and last-chance tour of Barrier 3</td>
<td>Jens Ellegaard, CEO</td>
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<tr>
<td>15:00-16:00</td>
<td>Beverages, snacks and farewell</td>
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WELCOME AND INTRODUCTION

Jens Ellegaard

Our new Barrier 3 has been built based on our mission, vision and our company values: Animal welfare, Quality, Collaboration and Respect. It has been designed to provide the best possible animal welfare to our minipigs and to secure our customers the highest standards of quality available with respect to health, genetics and physical conditions. Together with our staff and customers we have collaborated about improving the design of Barrier 3 to best accommodate the needs of people and minipigs. The design and size of Barrier 3 is based on respect for securing availability to our customers and respect to the environment. Today you are here to see the result.

ABSTRACTS

1. Prof. Dr. Henner Simianer, Georg-August-University Göttingen: Minipig history and genetic management

2. Dr. André H. Penninks, TNO: Immunotoxicity testing in the minipig

3. Dr. Helmut Ehall, Huntingdon Life Sciences: What you can see in the eye of the Ellegaard Göttingen Minipig

4. Adrian Zeltner, Ellegaard Göttingen Minipigs: Handling of a minipig (Demonstration)

5. Dr. Wolfgang Koch, Fraunhofer Institute of Toxicology: Inhalation toxicology: New validation

6. Dr. Arne Lund Jørgensen, Aarhus University and Ida Elisabeth Holm, Randers Hospital: Cloned Alzheimer's Model in Göttingen Minipigs

7. Christina Skytte, DVM & Henrik Søeborg, DVM, LAB Research: Convulsions in Minipigs

8. Dr. PhD. Aage Kristian Olsen Alstrup, Aarhus PET-Center: Anaesthesia and analgesia in Ellegaard Göttingen Minipigs


1. MINIPIG HISTORY AND GENETIC MANAGEMENT

Prof. Dr. Henner Simianer
Georg-August-University Göttingen, Germany

The Göttingen minipig was developed in the 1960s at the Institute of Animal Breeding and Genetics at the Georg-August-University Göttingen as a model for medical research. The breeding history is briefly reviewed including the various population bottlenecks the breed went through. The main challenge of the genetic management is to balance selection for desired traits (like fitness, small body size, smooth temperament etc.) with the maintenance of genetic diversity and the genetic uniformity of the different sub-populations. The strategy implemented to achieve these conflicting goals is described, and recent results on the genetic basis of relevant trait complexes like growth and temperament will be presented. The Göttingen minipig was recently analysed with novel high-throughput genotyping tools, allowing a high resolution analysis of the genomic architecture of the breed. First results of this analysis will be presented, which prove the actual success of the genetic management system implemented in the breed.
2. IMMUNOTOXICITY TESTING IN THE MINIPIG

Dr. André H. Penninks and G. van Mierlo
Experimental Immunology
TNO Quality of Life, Dept Toxicology and Applied Pharmacology
P.O. Box 360, Zeist, the Netherlands

As interest is growing for minipigs in non-clinical evaluation of pharmaceuticals their use in testing the potential immunotoxicity of pharmaceuticals was explored. In a subacute immunotoxicity study Göttingen Minipigs® were treated for 39 consecutive days with the immunosuppressive compounds Cyclosporin A (20 mg/kg/day) or Dexamethasone (0.4 mg/kg/day). At several time points, various quantitative (immuno)toxicological endpoints were analysed, such as clinical signs, body weight, haematology, and lymphocyte subset analysis in blood. At necropsy gross macroscopic changes, lymphoid organ weights, and histopathology of the collected lymphoid organs were further used as criteria for disclosing possible immunotoxicological effects. Potential effects on the function of the immune system were measured by the T cell-dependent antibody response (TDAR) to KLH, the delayed type hypersensitivity (DTH) response upon intradermal KLH injection, the ex vivo mitogen and KLH-induced lymphocyte proliferation, and the Natural Killer (NK)-cell activity in peripheral blood mononuclear cells (PBMC).

The results obtained will be discussed from which it is clear that the potential immunotoxicity of pharmaceuticals can be assessed in Göttingen Minipigs®.

3. WHAT YOU CAN SEE IN THE EYE OF THE ELLEGAARD GÖTTINGEN MINIPIG

Dr. Helmut Ehall
Dept. of Veterinary Services
Huntingdon Life Sciences

Exogenous or endogenous exposure to chemicals or therapeutic drugs results frequently in structural and functional alterations in the eye and central visual system. In many instances, alterations in visual function are the first and sometimes only clinical sign of toxicity. Ophthalmoscopic examinations are therefore an important part of most safety toxicology studies. In contrast to other organs, the eye is anatomically and physiologically very similar between laboratory species. The majority of ocular lesions in animals are breed-related or have at least a hereditary component to their pathogenesis. This underlines the importance to have an appreciation of the incidence of congenital and hereditary ocular lesions for any species used on a toxicology safety study.

While it is generally accepted that in comparison to other laboratory species ocular lesions are rare in minipigs, incidental background findings may still be detected during routine examination. This presentation summarizes ocular abnormalities observed during ophthalmic examinations performed in a selection of different age groups performed at the Ellegaard minipig breeding centre and during routine safety toxicology testing at Huntingdon Life Sciences.

4. HANDLING OF A MINIPIG (DEMONSTRATION)

Adrian Zeltner
Ellegaard Göttingen Minipigs A/S

This session will demonstrate selected application methods and the collection of a blood sample. With well prepared, confident staff and animals it will be more likely to perform the dosing relaxed and with a minimum of stress, which will contribute to the success of the study. When working with Minipigs you have to be aware that, unlike dogs, they are prey in their natural habitat. Most likely the pig will consider you as a predator and will react in a defensive or shy manner unless approached correctly. Although our Minipigs are socialized, we recommend that you use the acclimation period to socialize further and establish a relationship of trust and confidence.

You can do that by simply go and talk to them, hand feed and touch them or you can train with them the dosing procedures based on the positive reinforcement theory, e.g. clicker training.

Handling and dosing courses, as well as surgery courses, are held on a regular basis at the premises here, but a visit at your facility can be arranged too.

Do not hesitate to contact us if you have any questions regarding training of staff and Minipigs.
5. INHALATION TOXICOLOGY: NEW VALIDATION

Horst Windt, Heiko Kock, Wolfgang Koch
Fraunhofer ITEM, Hannover
Frank Runge, Ulrich Hübel
IPAS, Nycomed, Hamburg, Barsbüttel

Minipigs are attractive in toxicity testing because they show similarities to humans with respect to various anatomical and physiological parameters. However, little information is available on using minipigs in inhalation toxicology. In this presentation we show first data on the dosimetry of inhaled test particles. We focus on particle deposition in different regions of the minipig’s respiratory system and its dependence on particle size. Animals were exposed for one hour to chemically labelled test aerosols of different size. The compartments of the respiratory tract were dissected, and separately lyophilized and chemically digested. The concentrations of the tracer elements were determined by inductively coupled plasma mass spectrometry. Together with measured values of the respiratory minute volume and the concentration of the inhaled aerosol the regional deposition efficiency was calculated. Coarse particles larger than 5-8 µm are removed in the nostril sections and do not penetrate into the respiratory tract. Particles with aerodynamic diameter of 2 µm show high lung deposition: 20 % in the central lung region and 70 % in the peripheral airways.

6. CLONED ALZHEIMER’S MODEL IN GÖTTINGEN MINIPIGS

Arne Lund Jørgensen
Department of Human Genetics
Aarhus University;

Ida Elisabeth Holm
Lab. Experimental Neuropathology
Randers Hospital;

In an effort to develop a porcine model of Alzheimer’s disease we used random transgene insertion and handmade cloning to produce seven Göttingen minipigs (sows) carrying one copy of the cDNA of the neuronal variant of the human Amyloid Precursor Protein gene with the Swedish mutation (Kragh et al., 2009). Similar and robust levels of the transgene transcript have been detected in all pigs and high levels of expression, including high levels of the corresponding protein, in brain tissue from a 3 month old pig. A rough estimate predicts that accumulation of the A peptide in the brain, as a first sign of the disease may develop at the age 2 years and, accordingly, the next pig will be sacrificed in August 2009. Our protocol to monitor phenotypic development includes behavioral tests (olfaction and object recognition), imaging, and neuropathology. We have used the transgenic clones to produce 40 pigs. Of the 30 studied so far, 18 have inherited the transgene which is expressed.

7. CONVULSIONS IN MINIPIGS

Christina Skytte & Henrik Sæborg
LAB Research (Scantox)
Denmark

LAB Research (Scantox) has used the minipig in pre-clinical studies for 30 years and has obtained a significant experience with the use of this species. During the last 5 years we have experienced an increase in the number of juvenile minipig studies requested. In relation to a juvenile study we for the first time experienced two animals having a short lasting convulsive fit on several occasions, often in relation to feeding.

We contacted Ellegaard minipigs regarding these incidences and became aware that convulsions are seen as a low incidence background finding in the population of minipigs. Often the first incidences are seen at an early time point and the affected animals are terminated. No formal investigation of these convulsions had been performed.

Therefore, we have in cooperation with Ellegaard Minipigs initiated a project with the objective of investigating these convulsions. The objective of this project is to elucidate any possible pathological (including clinical pathology and histopathology) or genetic cause of these incidences of convulsions. The project will include 20 minipigs.
8. ANAESTHESIA AND ANALGESIA IN ELLEGAARD GÖTTINGEN MINIPIGS

Aage Kristian Olsen Alstrup, DVM, PhD
Aarhus PET-Center
Aarhus, Denmark
aage@pet.auh.dk

The Ellegaard Göttingen minipigs can be anaesthetized for several hours without higher risk of complications. Shortly, minipigs are sedated to avoid physical restraint and stress. Thereafter, an ear catheter is placed, and used for introduction of anaesthesia. After induction of anaesthesia, tracheal intubation is recommended, due to the low lung capacity of pigs, particular for long lasting anaesthesia. The anesthesia can be maintained by inhalation or injection anaesthesia. Pigs have a poor thermoregulation system, and therefore warming blankets should be used in order to prevent hypothermia. The successful anaesthesia of Göttingen minipigs should include sufficient analgesia, sedation and muscle relaxation. In this presentation I will give a short overview over anaesthesia and analgesia of the Göttingen minipig. A new publication of this topic will be presented.

9. SURGERY COURSES AT ELLEGAARD

Tony Webb
MRCVS, Scientific Consultant
Ellegaard Göttingen Minipigs A/S

Ellegaard Göttingen Minipigs A/S has contributed to education and training in anaesthesia and surgery of minipigs since 1996 by supporting courses and workshops. Historically these have been run in academic facilities roughly annually for large groups of participants. Since 2007, regular two day theoretical and practical courses have run at the breeding facility in Denmark. Group size is limited to 4 people which allows each participant to gain “hands on” experience in procedures while receiving a high level of personal attention from tutors. Courses are designed to cover the foundation techniques of minipig surgery which starts with preparation and planning, general anaesthesia, post-operative care and analgesia. Surgical techniques cover vascular access (catheters and vascular access ports) and telemetry implants. This syllabus contains the requirements of most minipig users and covers commonly used methods needed in pharmaceutical companies and contract research organisations. Courses are scheduled about every 12 weeks to allow rapid access to training. They can be run on demand at additional times to meet customers’ specific requirements and have also been run at customers’ facilities which can provide a cost effective solution for delivering training to a team of researchers.

MAY 2009 MINIPIGS WERE MOVED TO THEIR NEW HOME IN BARRIER 3

The staff made sure that the minipigs were moved with a strong focus on maintaining health status and securing good animal welfare. The staff made a great effort.

The minipigs arrive at their new surroundings in barrier 3, where they have now settled down.
Welcome

OPENING OF BARRIER 3
Minipig Symposium
Friday June 12th 2009

A few words from Jens Ellegaard, CEO

- Welcome
- Our history
- The story of Barrier 3
- Future prospects

Our history

Our history

Our history

Our history

Our history - Ellegaard Göttingen Minipigs
Our history

We believe in collaboration as a way of achieving the best results and good processes reaching these!

The story of barrier 3 - Collaboration

We believe in constant aiming at the best quality possible!

The story of barrier 3 - Quality

We believe in animal welfare and that it is an important responsibility, which we assume!

The story of barrier 3 - Animal welfare

The story of barrier 3 - Animal welfare
The story of barrier 3 - Animal welfare

We believe in sustainability and environmental responsibility and thus we put emphasis on environmental care!

The story of barrier 3 - Respect

Future prospects
Breeding history and genetic management of the Goettingen minipig

Prof. Dr. Henner Simianer
Animal Breeding and Genetics Group
Department of Animal Sciences
Georg-August-University Göttingen

The Goettingen minipig is unique among all other experimental animal species:
- it is a relatively young population: started in 1960, consolidated in 1992
- it is a relatively small population: the number of active breeders is ~ 1000
- the entire breeding population is located in just three physical locations (Denmark, Germany, USA)
- the entire population history is well documented: all matings are recorded back to the 1960s
- There is a common genetic management trying to maintain the integrity of the population together with improvements via selection

The Starting Phase - 1960 to 1969

Need for an experimental animal which is
- physiologically close to humans (skin, metabolism, nutrition)
- sufficiently large for certain treatments (e.g. surgery)
- phenotypically uniform
- modest in temperament (tame, not aggressive)
- easy to breed and to keep

1960 imported
- 3♂ and 2♀ Minnesota Minipigs (Hormel Institute, Austin, USA)
- 3♂ and 4♀ Vietnamese potbelly pigs (Wilhelma Zoo, Stuttgart) coloured population

1965 additional import
- 4♀ Vietnamese potbelly pigs (Zoo Friedrichsfelde, East Berlin) smaller, whiter

1965 - 1969
- Introgression of German Landrace ♂♂ to develop a white line
  - dominant white
  - weight
  - leanness

The Starting Phase - 1960 to 1969

Minnesota minipig (33%)
Vietnamese potbelly pig (60%)
German landrace (7%)

Introgression of Landrace genes using A.I. (Dr. Smidt)

F1-sow (Landrace x Vietnamese) x Minnesota boar

Segregating F2 animals
The Starting Phase - 1960 to 1969

Animals were kept under extensive conditions on the experimental farm of the University of Goettingen in Friedland
- high number of losses
- high proportion of low weight animals due to health problems
- unreliable records on the selection criterion weight at 5 months

1967 to 1972 first sanitation using hysterectomy
- newly built facilities in Dassel-Relliehausen (supported by the Volkswagen Foundation)
- technological cooperation with the Schaumann Hülsenberg pig breeding company

Closed population with 50 sows under SPF conditions
- 9 white and 8 coloured lines (paternal lines)
- Animals were sold directly to numerous institutions around the world

The Starting Phase - 1960 to 1969

The second sanitation - 1992 to now

1992 License contract with Ellegaard Göttingen Minipigs ApS, Dalmose, Denmark
- Ellegaard sets up a multiplier herd and has the exclusive world-wide right to sell Göttingen Minipigs
- University of Goettingen maintains the herd in Relliehausen and is allowed to sell directly to scientific institutions in Lower Saxony
- Genetic management of the entire population provided by Göttingen (P. Glodek, since 2001; H. Simianer)

Second sanitation 1992/1993
- hysterectomy of 35 sows in Denmark
- 280 primary SPF-piglets in Dalmose
- only white lines
- Sanitation and restocking of the Relliehausen herd with 30 SPF sows and 5 boars from Dalmose + additional lines through hysterectomy

Genetic management of the Goettingen minipig

Objectives
- Genetic uniformity
  A Goettingen minipig is a Goettingen minipig...
  Genetics and management
- Minimising inbreeding and random genetic drift
  Inbreeding depression and genetic defects
- Pursuing breeding goals
  → smaller size
  → smoother temperament
  → maintain fertility and disease resistance

Present population structure

Breeding for low body weight in Goettingen minipigs

F. Köhn, A.R. Sharif, H. Tauber, I. Malovrh, & H. Simianer

Figure 4: Changes in the current growth curve after 3 and 6 years of selection based on results from multiple trait models (SATR, selection at 150 days)
**Genetic management of the Goettingen minipig**

Genetic similarity within herds increases → Inbreeding

Genetic similarity between herds decreases → Genetic drift

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**New technologies**

**SNP-chips**: simultaneous genotyping of many evenly distributed markers in the genome for a reasonable price → better control of the genome → better selection tools

**Pilot study (M.sc. C. Gaerke)**

Illumina porcine SNP Chip with 62,163 SNPs after filtering 41,437 SNPs used

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192 animals genotyped

→ 5 discarded (call rate < 97%)

→ 187 animals analysed

**GMP** 134 Göttingen minipigs (Denmark, Germany)

**VPP** 4 Vietnamese potbelly pigs (Zoo Friedrichsfelde)

**MMP** 18 Minnesota minipigs + other minipigs (Austin, USA)

**GL** 14 German landrace pigs

**WB** 22 German wild boars

**Principal component analysis** — dissection of the genetic structure

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**First conclusions SNP study**

→ The Goettingen minipig is clearly separated from other pig breeds (including other minipigs)

→ Despite separation in sub-populations, the Goettingen minipig population is genetically coherent

→ Next steps/possibilities

→ Trying to dissect the genome according to the breed origin

→ Identification of causal segments of specific traits (e.g., body size)

→ Monitoring of genetic population structure

→ Genomic fingerprint of the Goettingen Minipig

→ SNP-based (genomic) selection

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**Summary**

→ Development of the Goettingen minipig started in the 1960s and since 1990 led to a consolidated population of increasing importance

→ Genetic management is necessary to minimise inbreeding and drift and maintain the genetic integrity of the multiplier herds

→ There is a conflict between breed development through selection and maintenance of the genetic uniformity of the breed — so far not too bad

→ Other new technologies (e.g., sequencing, transgenics, cloning) are just around the corner, potential benefits and risks need to be assessed

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Thank you for your attention
Why immunotoxicity testing in Göttingen Minipigs?

Growing interest exists in the use of minipigs as an alternative species to traditional non-rodent species for non-clinical toxicity studies, including immunotoxicity testing, of pharmaceuticals and food ingredients.

Why?

- Minipigs closely resemble man in many features of its anatomy, physiology, biochemistry and lifestyle, in particular the cardio-vascular system, skin and digestive tract, metabolic aspects.
- Because of these similarities the toxic effects of chemicals and drugs in pigs may resemble the effects in man more closely than do some other commonly used non-rodents and laboratory animals.

Aim of the project

To adapt and implement immune-toxicological endpoints, as routinely used in rodents, in Göttingen Minipigs®. Limited information is available in open literature on immunotoxicology assessment in minipigs.

- Pilot study: Adaptation/development of immunological endpoints
- Main study: Explore the possibilities/shortcomings of immunotoxicity testing in minipigs in a demonstrator project using two classical immunosuppressive compounds

Quantitative immunotoxicity testing*: Factors to consider in Standard (immuno)Toxicity Studies*

- Hematological changes (leukocytosis, lymphopenia)
- Alterations in immune system organ weights and/or histology (thymus, spleen, lymph nodes in route and distant from route of exposure, bone marrow, GALT, NALT, BALT)
- Changes in serum globulins might be an indication for changes in immunoglobulins (could call for immunoglobulin measurements)
- Increased incidence of infections
- Increased occurrence of tumors can be viewed as a sign of immunosuppression in the absence of other plausible causes such as genotoxicity, hormonal effects, or liver enzyme induction

*ICH S8 "Immunotoxicity studies for Human pharmaceuticals" (ICH step 4, in operation from April 2006)

Qualitative immunotoxicity testing*: Selection of additional immune function tests

- T-cell Dependent Antibody Response (PFC/KLH assay)
- Immunophenotyping of blood, spleen or lymph nodes
- Natural Killer Cell Activity Assays in blood or spleen
- Host-Resistance studies to bacteria, viruses, parasites or implanted tumors
- Macrophage/Neutrophil function assays
- Mitogen- or antigen-stimulated lymphocyte proliferation response
- CTL function
- MLR
- Specific cytokine production
- Cell-mediated Immunity (DTH)

Immunotoxicological study: assessment of immune toxicological endpoints in minipigs

- Groups
  - Vehicle
  - Cyclosporin A (20 mg/kg/day)
  - Dexamethasone (0.4 mg/kg/day)
- 4♂ and 4♀ per group
- Treatment:
  - days 0-38
  - oral intake (via food)

Clinical observations

- No treatment related clinical observations
- No effects on body weight of CsA treated animals and a reduction in body weight of dexamethasone-treated animals

Immunotoxicological study: time line

Start treatment

<table>
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<tr>
<th>Days after start treatment</th>
<th>Males</th>
<th>Females</th>
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<tr>
<td>0</td>
<td>12.5</td>
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</tr>
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</table>

- Proliferation assay
- Lymphocyte subset analysis
- NK activity assay

Serum primary IgM/IgG

Serum secondary IgM/IgG

Clinical observations

- No treatment related clinical observations
- No effects on body weight of CsA treated animals and a reduction in body weight of dexamethasone-treated animals
2. IMMUNOTOXICITY TESTING IN THE MINIPIG - DR. ANDRÉ H. PENNINKS

WBC differentiation in PBMC’s

Absolute and relative organ weights (males)

Absolute and relative organ weights (females)

Thymus

Lymphocyte subset analysis

Lymphocyte subset analysis in PBMC

Immunotoxicity testing

FUNCTIONAL ASSAYS

Natural Killer Cell activity

E:T 1:100
In vitro proliferation assay
(in vitro stimulation with medium)

Females
- Vehicle
- Cyclosporin A
- Dexamethasone

Males
- Vehicle
- Cyclosporin A
- Dexamethasone

In vitro proliferation assay
(in vitro stimulation with ConA)

Females
- Vehicle
- Cyclosporin A
- Dexamethasone

Males
- Vehicle
- Cyclosporin A
- Dexamethasone

In vitro proliferation assay
(in vitro stimulation with KLH)

Females
- Vehicle
- Cyclosporin A
- Dexamethasone

Males
- Vehicle
- Cyclosporin A
- Dexamethasone

KLH specific IgM responses

Females
- Vehicle
- Cyclosporin A
- Dexamethasone

Males
- Vehicle
- Cyclosporin A
- Dexamethasone

Delayed type hypersensitivity response:
Draize score

A. Erythema and eschar formation
- 0: No erythema
- 1: Very slight erythema (barely perceptible)
- 2: Well-defined erythema
- 3: Moderate to severe erythema
- 4: Severe erythema (beet redness); eschar formation

B. Oedema formation
- 0: No oedema
- 1: Very slight oedema (barely perceptible)
- 2: Slight oedema (edges of area well-defined by definite raising)
- 3: Moderate oedema (raised approximately 1 millimeter)
- 4: Severe oedema (raised more than 1 millimeter, extending beyond the area of exposure)

Conclusions

Quantitative endpoints of immunotoxicity testing, such as
• Hematological changes (leukocytosis, lymphopenia)
• Alterations in immune system organ weights and/or histology

Qualitative (functional) endpoints of immunotoxicity testing, such as
• Ex vivo lymphocyte proliferation
• NK cell activity
• Delayed Type Hypersensitivity responses
• Lymphocyte subset analysis (needs still some attention)
• A T cell-Dependent Antibody Response (TDAR) using KLH as antigen

were in general successfully implemented in minipigs.

Ellegaard Göttingen Minipigs

Acknowledgements

Geertje van Mierlo, project leader
Mary-lène de Zeeuw-Brouwer
Blanca Rappard
Marcel Schijf
Marieke Slotboom-Visser
Marlies Otto
Marko Appel
And many others from the animal house, histopathology department etc
What You Can See In The Eye Of The Ellegaard Göttingen Minipig

Helmut Ehall
Huntingdon Life Sciences
Dept of Veterinary Services

Ophthalmic Examinations & Safety Assessment

- Alterations in visual function are often the first and sometimes only clinical sign of toxicity
- Simple anatomy
- Different types of tissue directly visible and/or assessable
- Ophthalmoscope
- Slitlamp Biomicroscope

Hermann von Helmholtz
1821 - 1894

If an optician should try to sell me an instrument possessing such faults, I would feel justified in using the most severe language with regard to the carelessness of his work and return the instrument under protest.
THE LENS

- Focuses light onto the retina
- must be transparent
- must be stable
- must be able to change its size

Heterochromia Iridis

Heterochromia Irides

Iris Coloboma

Microphakia
3. WHAT YOU CAN SEE IN THE EYE OF THE ELLERGAARD GÖTTINGEN MINIPIG – DR. HELMUT EHALL

Hyaloid Remnant

Persistent Hyaloid Artery

Posterior Subcapsular Cataract

What’s normal?

Cataract - Cortical

Fundus Variations

3. WhaT yOu can see in The eye Of The ellegaard göTTingen Minipig – dr. helMuT ehall
3. WHAT YOU CAN SEE IN THE EYE OF THE ELLEGAARD GÖTTINGEN MINIPIG – DR. HELMUT EHAU
**Inhalation Experiments with Minipigs**
- Particle deposition in the respiratory tract -

Horst Windt, Heiko Kock, W. Koch, Fraunhofer ITEM
Frank Runge, Ulrich Hübel, IPAS, Nycomed

Elegant Symposium, 12. June, 2009

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**Non-rodent exposure system**

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**Minipig PK-study with Verapamil**

Koch et al., 2001

---

**Outline of the new study**

Determination of regional particle deposition efficiency

- Development of test aerosol
- Pre-study with one animal
- Dissection of relevant compartments of the respiratory tract
- Chemical evaluation of the test material in the biol. matrix
- Main study with 4 animals

---

**Test particles**

Using 3 different chemical tracers in 3 different size distributions

Spray-drying process

- Nebulization of aqueous nano-suspension of rare earth elements: Er₂O₃, Sm₂O₃, Y₂O₃
- Evaporation of the water
- Inhalation of dry aerosol particles (1 hour)
Test particles

Exposure

Exposure concentration

Minute volume

Sample preparation and chemical analysis

Data evaluation: chemical mass balance method

Regional deposition matrix:
Regional deposition matrix:

- Nostril
- Nasal mucosa, pharynx, larynx
- Trachea, bronchi
- Peripheral lung section

Deposition efficiency [-]

Aerodyn. particle diameter [µm]

Deposition efficiency [-]

Aerodyn. particle diameter [µm]

Deposition efficiency [-]

Aerodyn. particle diameter [µm]

Conclusions

- Chemical mass balance method suitable to measure regional particle deposition in the minipig.
- Particles larger than approximately 5 µm are effectively removed in the nostril region.
- Particles in the size range between 0.5 and 3 µm are deposited mainly in the lung periphery of the animal.
- Results consistent with assumptions made for the Verapamil study (MMAD 1.5 µm).

Next steps

- Refinement of test aerosol size distribution
- Exposure of 4 animals in parallel
- Recovery of material from snout and nostril
- Extended statistical data analysis
Convulsions in Minipigs

Christina Skytte, DVM
Henrik Søeborg, DVM

Convulsions in minipigs

- According to Ellegaard minipigs, convulsions are seen as a low incidence background finding in the population of minipigs.
- Most incidences are seen at an early age (2-4 month old) and the affected animals are terminated.
- No formal investigation of these convulsions had been performed up to that point.
- Therefore 7 months ago, we have in cooperation with Ellegaard Minipigs initiated a project with the objective of investigating these convulsions.

Project outline

- The objective of this project is to elucidate any possible pathological or genetic cause of these incidences of convulsions.
- When it is completed the project will include 20 minipigs.
- After a convulsion has been observed at Ellegaard Minipigs facility, a clinical description of the incidence is made. Furthermore, body temperature is recorded and blood samples are collected for clinical pathology.

Possible causes of convulsions

- Many different causes of convulsions:
  - Injury/trauma to the head
  - Tumors
  - Infections (meningitis/encephalitis)
  - High fever
  - Heat stroke
  - Diabetes
  - Genetics
  - Imbalance in blood constituents eg electrolytes (NaCl glucose etc)
  - Epilepsia like conditions
  - And more
- Hope to find some evidence hereof through the mentioned investigations.

Results

Clinical description

- Clinical description of the convulsions:
  - In relation to feeding, the animal gets a convulsive fit often with clonic cramps.
  - The convulsion lasts in average 30 sec to 1 minute.
  - The animal recovers for a similar period. Therafter, no clinical signs are seen.
  - On most occasions, the animal continues to eat.

Haematology & clinical chemistry

- All values for haematology and clinical chemistry are within normal range for minipigs.
- All organ enzymes are within normal range.
- All levels of electrolytes are within normal range.
- No signs of dehydration are observed.
- No signs of hypoglycemia are observed.
Possible infections?

- The body temperature has been measured in 8 of the 12 animals as soon as possible after the convulsions were observed.

- In 6 animals, an increased body temperature in a range of 38.5 – 39.8 degree Celsius was observed.

- The total white blood cells and absolute values of neutrophils were within our historical range. However, in 2 animals the percent neutrophils were slightly above our historical range.

Possible infection?

- Apart from the incidences of convulsions, the animals have not shown abnormal behavior.

- No observations were made regarding reduced food consumption.

- Based on these results to date there is no clear indication of an infectious cause for these convulsions.

Results - Body weight

- The incidences seen at LAB Research (Scantox) were in both cases the smallest animals in the study.

- Looking at the data from Ellegaard Minipigs, most animals seem to weigh below the average body weight for the specific age.

- The relation between this lower average body weight and the convulsions is at present not clear.

Background

- Status
  - As mentioned, 12 minipigs have been delivered for necropsy
  - No notable findings reported at necropsy
  - 11 brains have been evaluated histologically

- Histology
  - Full standard histologic examination
  - Extended evaluation of the central nervous system (CNS)

Extended evaluation of the CNS

- The brain will be removed from the skull with as much of the olfactory bulbs intact as possible.

- The brain is cut in halves and the first slice is made through the middle of the olfactory bulb. It determines the angle and location of the following slices.

- As the main focus of histopathology is the brain - we have implemented a new procedure for the removal and trimming of the brain and for the amount of slides to be evaluated histologically.

- Following the first slice serial coronal sections 3 mm apart will be made consecutively to yield approximately 19 slices.

- Of the 19 slices 12 are chosen and trimmed to yield 13 - 14 slides depending on the size of the brain.

- This gives a broad overview of the brain homogenous and still varying due to angle and depth of cut.
Microscopic evaluation

- Where are we looking
  - In principle, all over the brain as the misfiring of neurons can happen in all parts of the brain and propagate
  - Special focus is on the frontal and temporal part of the lobes seen in man to occur frequently in young individuals
- What are we looking for
  - Damaged neurons
  - Blurring of the laminar structure in cortex
  - Decreased/increased cellularity in grey/white matter respectively
  - Increased numbers of glial cells
  - Others (e.g., vacuolation of neurons)

Pathology results

- Result
  - So far there are no apparent pathological changes to explain the convulsive seizures observed in these animals
- Further examination
  - Slides of specific areas in the brain from the left intact side of the brain
  - Staining with Flourojade B for damaged neurons could be a possibility

Further investigations

- The genetic relationship between the affected animals will be investigated by Ellegaard Minipigs, these results are still pending.
- No clear conclusions can be made before this information and the results from the histopathology on remaining organs are available.
- In the current project, no EEG recordings of the brain have been performed. EEG recordings might give further information about these convulsions.

Treating of minipigs prior to anaesthesia

Acclimatisation
1-2 weeks

Clinical examination
- Respiration: 10-30 /min
- Heart rate: 68-98 /min
- Body temperature: 37-38 °C

Fasting
- Young and adult: 6-12 hours
- Neonates: 3 hours
- Elective surgery: 12-24 hours
Premedication of Göttingen minipigs

- Atropine 0.05 mg/kg Only anticolinergic effect
- Azeperone 6 mg/kg Moderate sedation
- Azepramazine 0.3 mg/kg
- Diazepam 1 mg/kg God muscle relaxation
- Midazolam + ketamine 1+10 mg/kg Useful for scanning
- Pig Zoletil mixture 1/15 ml/kg Deep sedation
  - Pig Zoletil mixture: 1 bottle of Zoletil + 6.25 ml xylazine (20 mg/ml) + 1.25 ml ketamine (100 mg/ml) + 2.5 ml butorphanol (10 mg/ml)

(All drugs given IM)

Ear vein catheters and intubation

Ear vein catheter
21G Venflon

Induction of anaesthesia (IV)
- Propofol 3 mg/kg
- Thiopentone 4 mg/kg
- Ketamine-Midazolam 1+5 mg/kg

Intubation
- Tube size: piglets 3.0-4.0 mm
  - 10-15 kg 5.0 mm
  - Adults 5.0-7.0 mm

Maintaining of anaesthesia by inhalation

<table>
<thead>
<tr>
<th>Drug</th>
<th>MAC-values</th>
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<tbody>
<tr>
<td>Halothane</td>
<td>0.9 - 1.3 %</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.5 - 2.0 %</td>
</tr>
<tr>
<td>Desflurane</td>
<td>10 %</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>2.0 - 2.7 %</td>
</tr>
<tr>
<td>Enflurane</td>
<td>1.7 %</td>
</tr>
<tr>
<td>N2O</td>
<td>162 - 277 %</td>
</tr>
</tbody>
</table>

Maintaining of anaesthesia by injection

<table>
<thead>
<tr>
<th>Drug</th>
<th>Infusion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>IV 4-10 mg/kg/h</td>
</tr>
<tr>
<td>Diazepam</td>
<td>IM 1-2 mg/kg</td>
</tr>
<tr>
<td>Ketamine</td>
<td>IM 10-18 mg/kg</td>
</tr>
<tr>
<td>Xylazine</td>
<td>IM 2 mg/kg</td>
</tr>
<tr>
<td>Ketamine</td>
<td>IM 15 mg/kg</td>
</tr>
<tr>
<td>Xylazine</td>
<td>IM 2 mg/kg</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>IM 220 µg/kg</td>
</tr>
<tr>
<td>Ketamine</td>
<td>IM 5 mg/kg</td>
</tr>
</tbody>
</table>

Supplementary analgesia for painful surgery

<table>
<thead>
<tr>
<th>Drug</th>
<th>Infusion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfentanil</td>
<td>IV 6 µg/kg/hour</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>IV 30-100 µg/kg/hour</td>
</tr>
<tr>
<td>Remifentanil</td>
<td>IV 30-60 µg/kg/hour</td>
</tr>
<tr>
<td>Sufentanil</td>
<td>IV 15-30 µg/kg/hour</td>
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Monitoring during anaesthesia

Continuously:
- Heart rate
- Blood pressure
- Body temperature
- Pulse oximetry
- ETCO₂

Every 30-60 minutes:
- Blood gases (PaCO₂, PaO₂, pH)
- Blood glucose
- Reflexes

Recovery after anaesthesia

- Room temperature should be 20-25 °C
- Monitoring every 5 min until extubation
- Extubation when strong swallowing reflex is apparent
- Food and water when the minipig is fully conscious
- Postoperative analgesia

Postoperative analgesia

- Opioids
  - Buprenorphine 5-20 µg/kg 6-12 hourly
  - Butorphanol 0.1-0.4 mg/kg 4-6 hourly
  - Morphine 0.2 mg/kg 4 hourly
  - Pethidin 2 mg/kg 2-4 hourly
- NSAID
  - Acetylsalicylic acid 10-20 mg/kg 4 hourly
  - Carprofen 1-2 mg/kg daily
  - Flunixin 1 mg/kg daily
  - Ketoprofen 3 mg/kg daily
Background

- Ellegaard Göttingen Minipigs A/S has supported training in anaesthesia and surgery since 1996 (Odense)
- Workshops at universities and conference satellites
  - KVL modular MSc course - Copenhagen
  - SCANLAS meetings

Some drawbacks of workshops

- These provided high quality education but
- Training was infrequent (12-24 month intervals)
- Catered for large groups
  - Difficult to give “hands on” experience for all participants

Ellegaard courses

- Started in February 2007
- 0.5 day theory
- 1.0 day “hands on practical”
- Small participant groups (4 maximum)
- Scheduled at 12 week intervals
  - See website for dates
  - Additional courses delivered on request
- Licensed by Danish Ministry of Justice

Course venues

- Run at breeding facility in Dalmose
  - Combined with supplier visit, handling and dosing training (half day)
- Tailored courses may be run at customer facilities
  - Cost effective (travel costs and time)
  - Allows education of larger groups

COURSE CONTENT

Content (1) anaesthesia

- Preparation & planning
- General anaesthesia
- Analgesia – pain & distress

Content (2) vascular access

- Short term: Arrow™ catheter
- Long term: Catheter implants & Vascular access port
Content (3) telemetry

Data sciences
D70 PCT
(BP, ECG & temp)

Content (4)

Abdominal procedures
• Bile duct
• Portal vein
• GI cannulas

“Special requests” – CSF access, lymphatic catheters, wound models

Wound closure

Concluding points

• For more information on surgery and anaesthesia training contact Ellegaard Minipigs A/S

• We are happy to assist with arranging access to training in advanced anaesthesia and surgical techniques
We offer various types of equipment that can make your work with minipigs easier. You are welcome to contact us if you need any auxiliary equipment, and if you would like a demonstration of the equipment, you can sign up for one of our Handling and dosing courses.

**AUXILIARY EQUIPMENT**

Bite bar

Sling frame

Restraint chair for gavaging

Restraint bench for blood sampling

Minipig scale
Evaluation of a new hybrid technique for closure of muscular ventricular septal defects in a longterm setting

Cytoplasmic inheritance of transplantation antigens in animals produced by nuclear transfer

Mesenchymal stem cells prolong composite tissue allotransplant survival in a swine model

Experimental investigation of encephalomyosynangiosis using gyrencephalic brain of the miniature pig: histopathological evaluation of dynamic reconstruction of vessels for functional anastomosis. Laboratory investigation

Correlation of donor leukocyte chimerism with pulmonary allograft survival after immunosuppressive drug withdrawal in a porcine model

Serial assessment of left ventricular remodeling and function by echo-tissue Doppler imaging after myocardial infarction in streptozotocin-induced diabetic swine

Nutritional model of steatohepatitis and metabolic syndrome in the Ossabaw miniature swine

Upregulation of CD59: potential mechanism of accommodation in a large animal model

Treatment of a uterine adenocarcinoma in a miniature pig by ovariohysterectomy

Characterization of peri-infarct zone heterogeneity by contrast-enhanced multidetector computed tomography: a comparison with magnetic resonance imaging

Interindividual differences in o,p’-DDD enantiomer kinetics examined in Göttingen minipigs

Laparoscopic adrenalectomy for beginners without open counterpart experience: initial results under staged training

Generation of induced pluripotent stem cell lines from Tibetan miniature pig

A novel hybrid method for creating a porcine model of cyanotic congenital heart defect with decreased pulmonary blood flow

Effect of thrombin fragment (TP508) on myocardial ischemia-reperfusion injury in hypercholesterolemic pigs

Six-month angiographic study of immediate autologous bone marrow mononuclear cell implantation on acute anterior wall myocardial infarction using a mini-pig model

A novel and stable “two-hit” acute lung injury model induced by oleic acid in piglets
NEW ARTICLES ABOUT MINIPIGS

- Stem cells from deciduous tooth repair mandibular defect in swine

- Comparative healing response after sirolimus- and paclitaxel-eluting stent implantation in a pig model of restenosis.

- Porcine models of coronary atherosclerosis and vulnerable plaque for imaging and interventional research

ELLEGAARD GÖTTINGEN MINIPIGS DVDS AVAILABLE:

The Göttingen Minipig – Handling and dosing.

This DVD is available in German as well as French and English.
A unique tool for those who work with the Göttingen Minipig.
Price each € 90 excl. shipping and handling.

The Göttingen Minipig – Histology

This DVD allows users to familiarize themselves with the normal histology of the Göttingen Minipig.
Price each € 65 excl. shipping and handling.

Please contact us for further information at ellegaard@minipigs.com or phone +45 5818 5818.

IF YOU RECEIVE ELLEGAARD GÖTTINGEN MINIPIGS A/S NEWSLETTER BY MAIL...

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To correct mailing list problems, please send an e-mail to ellegaard@minipigs.dk or write to us at this address:

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Soroe Landevej 302
DK-4261 Dalmose

Thank you

PLEASE NOTE:
Ellegaard Göttingen Minipigs A/S will not release or give away a subscriber’s e-mail address, name or any other information provided.
ANNUAL MEETINGS OF THE MINIPIG RESEARCH FORUM

In October, the Minipig Research Forum will be hosting both a European and a North American meeting. At both meetings relevant and interesting topics will be presented and discussed.

On 28-29 October 2009, the annual meeting of the Minipig Research Forum in Europe will be held in Cannes, France. The topics will include surgery and surgery techniques; sampling & dosing techniques; the cardiovascular system and clinical pathology.

On 15-16 October 2009, the meeting of MRF North America will be held in Virginia, USA. At this meeting, the topics include surgery in the minipig; the use of minipigs for FDA-regulated products; the minipig in cardiovascular research; dermal toxicity testing in the minipig; and housing, husbandry and enrichment.

The preliminary programme is available at www.minipigresearchforum.org, where you can also register for the meetings.

YOU STILL HAVE THE CHANCE TO REGISTER FOR THE MEETINGS!

SURGERY AND ANAESTHESIA COURSE PLAN 2009-2010

So far four courses have been planned for the rest of 2009 and the first six months of 2010

2009:  
7–8 October
7-8 September
7-8 September
13-15 September
13-16 September
16-17 September
15-16 October
28-29 October
3-4 November
1-2 December

Additional courses may be arranged for at least 3 persons. Therefore, if you need a surgery course, don’t hesitate to ask!

Courses are tailored to the specific needs of the participants. When registering for a course you will be asked to fill out a questionnaire so that we can ensure that course content and pace match your training requirements.

Visit www.minipigs.com and learn more.

MEETING CALENDAR

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<thead>
<tr>
<th>NAME</th>
<th>DATE</th>
<th>PLACE</th>
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</thead>
<tbody>
<tr>
<td>ETS</td>
<td>7-8 September</td>
<td>Arles, France</td>
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<tr>
<td>SGV/ESLAV/SAVIR/VAWW Meeting 2009</td>
<td>7-8 September</td>
<td>Zürich, Switzerland</td>
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<td>GV-Solas</td>
<td>13-15 September</td>
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<td>SPS</td>
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<td>Strasbourg, France</td>
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<td>MRF-Europe</td>
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<td>ACT</td>
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<td>Blankenberge, Belgium</td>
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