

MARK YOUR CALENDAR!

**The Opening of Barrier 3:
Friday 12 June 2009**



**Reception, scientific programme
and guided tour of our new facility**

The scientific programme will include:

- Immunotoxicity in Göttingen Minipigs
- History and genetic background of Göttingen Minipigs
- Background data on seizures
- Photo toxicity
- Inhalation toxicology
- Cloned Alzheimer's model

**More information will follow,
but you are welcome to contact us
if you are not already in our database**

Minipig Research Forum
Two meetings in 2009
USA & Europe
MRF-Europe: 28 - 29th October
MRF-USA: October
Register & learn more at
www.minipigresearchforum.org



Minipigs

Biological products

Auxiliary equipment

Training & Courses

DEAR READER,

Preparing for this leading article, I found myself looking back at 2008, which was a sound and prosperous year for Ellegaard Göttingen Minipigs. I want to share this with you, because it naturally has some important perspectives that might interest you as well; our solidity is strong and healthy and we are working on a sound foundation, which secures our status as a solid business partner – even during the present global economic challenges. We are also both willing and able to continue our dedicated efforts to generate more background information about the Göttingen Minipig, background information that we hope you will find interesting and useful. And finally we continue to enlarge our organization to provide even better service to you in the future. We are working strategically with our human resources – with a special focus on education and training and the promotion of hidden skills we all hold. We also continue to extract practical knowledge of working with the minipigs from our qualified caretakers and forward this knowledge and know-how to you.

The construction of our new “Barrier 3” is also going well. The work has progressed as planned – even if we have been challenged by the Danish winter. By Christmas the building had been raised, and presently all interior work is done where painters are working alongside ventilation experts, electricians, plumbers, etc. Most of our staff have visited the construction site, and broad smiles have radiated from under their hardhats as they’ve seen their own ideas and input integrated into this new state-of-the-art barrier. We expect the construction of Barrier 3 to be finished by late April. Then we will initiate a thorough cleaning and disinfection process to meet our high standards for the interior environment of our barriers. Our production this spring is planned so we can meet the increasing market demand and also establish a new breeding herd of microbiologically defined Göttingen Minipigs for Barrier 3. We look forward to opening the barrier for this new herd.

We also look forward to showing you our new facilities, and I am therefore sincerely pleased to invite you to a grand opening of Barrier 3. We will be glad to welcome you here on Friday 12 June! Please see the front page for more information.

In 2008, we experienced an increased demand for pregnant sows for juvenile studies, and sows accompanied with boars for reproduction toxicology studies. As always, we do our utmost to meet all market inquiries and have adapted our production to this increasing demand. If you have a similar special demand, such as minipigs for juvenile or reproduction toxicology studies, please contact us – we will be delighted to assist you!

For some time now, we have been looking for a reliable committed business partner in Japan, and I am therefore pleased to announce our cooperation with Oriental Yeast Company in Japan. Oriental Yeast Company has a profile that meets our requirements for experience, high scientific standards and a solid reputation. Together we are aiming to establish a breeding unit for the microbiologically defined Göttingen Minipigs in Japan which will live up to our “Ellegaard Standard”, and which can then supply the Japanese market. The first step in our collaboration will be seeding the market with regular imports of minipigs from Denmark to Japan. When the market has grown to a steady demand for Göttingen Minipigs a breeding unit will be built. We believe our growth is closely connected to our focus on quality in both products and services and, although we are busy expanding on many levels, we do our best to continue delivering “Ellegaard Quality” in both products and services. I hope this newsletter manifests exactly that!

Jens Ellegaard
Managing Director

MINIPIG CASE STUDIES

Minipigs¹ have been used in biomedical research for several decades and have proven themselves to be a valuable animal model in basic research and in the development of new medicines. In the latter they are used extensively in regulatory toxicity testing as part of the safety assessment supporting first-human-dose studies. This article is a non-exhaustive list of marketed drug products where minipigs have been used in the non-clinical programme. A review of FDA and EMEA approval packages is ongoing and will describe in much greater detail the individual non-clinical development programmes. The review will be published at a later date.

Publications in peer-reviewed scientific journals are an integral part of basic research which ensures a steady influx of new knowledge about minipigs and their relevance in various fields of research. Lessons learned in basic research are often applied in the development of new medicines. The flow of information from the pharmaceutical industry to the scientific community is often limited due to intellectual property rights and competitive considerations. One source of information relating to the use of minipigs in developing new medicines comprises the FDA and EMEA approval packages of marketed drug products. These contain documentation submitted to regulatory authorities on which marketing authorization is based and offer a unique, informative view of the use of minipigs in the development of new medicines, both prescription and over-the-counter (OTC) drugs.

A non-exhaustive list of marketed drugs sorted by trade name is shown in Table 1.

The number of marketed drug products cover a number of indications and drug classes (see Table 2) both of which are lower in number than the number of marketed products. This is because there are multiple products within the same indication and/or drug class.

Historically minipigs have been used extensively in dermal toxicity testing, a fact reflected in the number of products with dermal indications (see table 2). The second-largest single group is ocular indications, followed by cardiovascular. In recent years there has been a shift in that an increasing number of compounds for oral administering are being tested in minipigs, but given the development times for new medicines it is likely to be some years before this change is reflected in the indications.

As stated above, a thorough review of the FDA and EMEA approval packages probing the use of minipigs in non-clinical development programmes is being prepared and will be published at a later date. Should you have any questions about the case studies, please feel free to contact Niels-Christian Ganderup (ncg@minipigs.dk).

¹ The term “minipig” denotes all types/strains of minipig, i.e. not the Göttingen Minipig exclusively.

Actonel, Altabax, Altargo, Clobex, Cordarone, Coreg, Dovobet, Dovonex, DUAC Topical Gel, Elidel, Fosamax Fuzeon, Levemir (insulin detemir), Metvixia, Miacalcin, Mirapex, Mobic, Myoview, Neupro, Olux-E, Protopic, Septocaine, Solagé, Solataze, SPF 20 Water Resistance Sunscreen Lotion, Tazorac, Temovate E, Tri-Luma, UV Expert 15 Sunscreen, Vaniga, Vanos, Venofer, Voltaren.

Table 1 Market drugs (trade names) where minipigs have been used in a non-clinical programme. Names are listed alphabetically; some compounds are marketed under several brand names.

Target Organ/System	Indications	Drug Classes
Topical (dermal)	<ul style="list-style-type: none"> • Acne vulgaris • Dermatitis, atopic • Hirsutism • Hypopigmentation, facial • Impetigo • Keratosis, actinic • Lentigines, senile & solar • Melasma • Prevention of sunburn due to sun exposure • Psoriasis 	<ul style="list-style-type: none"> • Antibiotics, lincosamides • Anti-infectives, topical • Anti-inflammatory, • Corticosteroids, topical • Depigmenting agents • Depilatory agents • Dermatologic • Immunosuppressant • Keratolytics • Photosensitizers • Retinoids • Sunscreen products
Ocular	<ul style="list-style-type: none"> • Choroidal neovascularization • Histoplasmosis, ocular • Inflammation, ophthalmic, postoperative • Macular degeneration • Myopia, pathologic • Pain, ophthalmic • Photophobia, postoperative 	<ul style="list-style-type: none"> • Ophthalmics
Other	<ul style="list-style-type: none"> • Hypertension, essential • Imaging, cardiac • Infection, human immunodeficiency virus • Infection, Trypanosoma brucei gambiense 	<ul style="list-style-type: none"> • Antiprotozoals • Antivirals • Diagnostics, radiopharmaceuticals • Fusion inhibitors • Vasodilators
Cardiovascular	<ul style="list-style-type: none"> • Arrhythmia, ventricular • Fibrillation, ventricular • Heart failure, congestive • Tachycardia, ventricular 	<ul style="list-style-type: none"> • Antiadrenergics, beta blocking • Antiarrhythmics, class III
Anaesthesia / analgesia	<ul style="list-style-type: none"> • Anaesthesia (infiltration, local, regional) • Pain, mild to moderate • Dysmenorrhoea 	<ul style="list-style-type: none"> • Analgesics, non-narcotic • Anaesthetics, local amide-type
Bone (calcium metabolism)	<ul style="list-style-type: none"> • Hypercalcaemia • Osteoporosis • Paget's disease 	<ul style="list-style-type: none"> • Bisphosphonates • Hormone/hormone modifiers
Arthritides	<ul style="list-style-type: none"> • Ankylosing spondylitis • Arthritis, osteoarthritis & rheumatoid 	<ul style="list-style-type: none"> • Analgesics, non-narcotic • Nonsteroidal anti-inflammatory drugs
Central nervous system	<ul style="list-style-type: none"> • Parkinson's disease, early-stage idiopathic 	<ul style="list-style-type: none"> • Antiparkinson agents • Dopaminergics
Metabolic	<ul style="list-style-type: none"> • Diabetes mellitus 	<ul style="list-style-type: none"> • Long acting, soluble human insulin analogue of recombinant DNA origin.
Transplantation	<ul style="list-style-type: none"> • Rejection, heart, liver, renal transplant, prophylaxis 	<ul style="list-style-type: none"> • Immunosuppressives

Table 2 indications and drug classes: the marketed drugs (see Table 1) cover a number of indications and drug classes. They are grouped according to the number of target organ/system based on number of indications which fall into each category. Indications and drug classes are sorted alphabetically

NON-SURGICAL MINIPIG CATHETERIZATION: USING THE ARROW CATHETER

Intravenous injection and especially serial blood sampling of the Göttingen Minipig may prove difficult, since large blood vessels like the jugular vein are not clearly visible and the ear veins are small.

Blood sampling is often performed by placing the pig in a V-trough to collect blood from the precaval sinus. This procedure requires specially trained personnel to ensure that the pig is not stressed, yet the method is inexpedient if frequent samples have to be taken from the pig over a short period of time.

Also, this method is not favourable if urine and faeces have to be collected quantitatively – in addition to the blood samples, and if the pigs have to be housed in metabolic cages during a study.

An alternative blood sampling or intravenous injection method is to implant a catheter surgically. The various types of catheters available include a specialized catheter known as a Vascular Access Port (VAP) where the catheter is connected to a subcutaneously placed reservoir.

An implanted catheter may also be connected to an automated blood sampler to obtain samples without having to handle the pig.

One disadvantage of these methods is that the pig has to undergo surgery to find and catheterize the vein selected.

To avoid a surgical procedure, an alternative is to implant the ARROW® catheter using the Seldinger technique. The catheter can remain open for up to 14 days and has the advantage that it is easy to replace if it gets clogged. This is done by introducing a new guide wire, removing the catheter and replacing it with a new catheter.

If you would like further details on how to use the ARROW® catheter in the minipig, please e-mail us at ellegaard@minipigs.dk.

MEETING CALENDAR

NAME	DATE	PLACE
SOT ToxExpo	15-19 March	Baltimore, USA
BTS	23 March	University of Warwick, UK
IAT	31 March – 3 April	Europe
Scand-LAS	23-25 April	Ystad, Sweden
AGAH	28-29 April	Lyon, France
AFSTAL	3-5 June	Nantes, France
ETS	6-10 September	Arles, France
SGV/ESLAV/SAVIR/VAWV Meeting 2009	7-8 September	Zürich, Switzerland
GV-Solas	13-15 September	Vienna, Austria
Eurotox	13-17 September	Dresden, Germany
SPS	15-18 September	Strasbourg, France
ACT	1-4 November	Palm Springs, USA

THE MINIPIG AS A MODEL IN JUVENILE TOXICOLOGY

Abstract from “Non clinical pediatric testing requirements – principles and practice”.
Chapter 14: Use of the swine pediatric model. Book soon to be published by Wiley.

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One-week old Göttingen minipig. Drawing by Mariline Leroy, Study Director, MDS Drug Safety Assessment

The popularity of the minipig model for the safety testing of drugs has continued to increase over recent years. Until now, juvenile minipigs have rarely been used for the safety testing of pediatric drugs. This is bound to change, however, as the advantages of the minipig for the safety testing of pediatric drugs become more widely known.

A pediatric assessment is now a required component of all new drug applications in the USA and Europe. In the USA, the Pediatric Research Equity Act (PREA) requires a pediatric assessment as part of all New Drug Applications (NDA) and biologics licensing applications (or supplements to applications) unless the applicant has obtained a waiver or deferral. Similar legislation exists in Europe. Both the FDA and the EMEA have issued guidance on the performance of non-clinical safety studies in juvenile animals.

Under most circumstances, the rat is the preferred species for juvenile safety testing: it is certainly the most convenient species and, for many aspects of post-natal development, is also the most studied. For some drugs, however, rodent species may not be an appropriate model, and larger species have to be used. Also, the FDA sometimes requests juvenile studies in both a rodent and a non-rodent species. The swine pediatric model should always be considered as a potential species for such studies, in view of its close

metabolic similarity to man and the ethical and practical advantages over other non-rodent species for this type of study.

The main disadvantage of even miniature breeds of pig in safety testing is the animals' large size at maturity, requiring large quantities of drug. This drawback does not hold true for juvenile studies, however, because the minipig attains sexual maturity much earlier than the dog and at a relatively low body weight (about 9 kg). This rapid maturation allows all of the stages of post-natal development to be covered in a shorter period than in other non-rodent species, with the reduced study duration providing economies in both time and test item requirement. Also, many of the required technical procedures (blood sampling, dosing, cross fostering, catheter implantation, etc.) require less skill and are less traumatic in the juvenile minipig than in the dog.

A typical juvenile study comprises five male and five female piglets in each of four groups (three treated plus control). More animals are required if some piglets are to be retained for a post-dosing follow-up phase (e.g., five piglets of each sex terminated at the end of treatment, three of each sex retained for follow-up to sexual maturity). The duration and timing of the treatment period is defined according to the age at which children will be given the drug and the corresponding stage of post-natal development of the minipig. A whole-litter design, in which the whole litter is treated at the same dose level, is usually preferred for main studies. The risk of cross-contamination between the pups is high when a split-litter design is used, but the increased statistical power provided by performing paired comparisons within the litter may outweigh this drawback.

The juvenile minipig model is particularly well suited for evaluating the effects of drugs on the development of the central nervous system, the gastro-intestinal tract, the reproductive system (male and female) and the immune system. More research is nonetheless still needed to improve our knowledge of the patterns of post-natal morphological and functional development of all laboratory species.

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There is growing interest in the use of minipigs as an alternative species to traditional non-rodent species for non-clinical studies of pharmaceuticals. In order to acquire experience in testing the potential immunotoxicity of pharmaceuticals in minipigs, in a pilot study we developed some assays to test for immunotoxicological endpoints in Göttingen Minipigs®. The various assays developed were subsequently implemented in a subacute immunotoxicity study in which Göttingen Minipigs® were treated for 39 consecutive days with the classical 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 in these animals, such as clinical signs, body weight, haematology, lymphocyte subset analysis and the Natural Killer (NK)-cell activity in peripheral blood mononuclear cells (PBMC), and ex vivo mitogen and KLH-induced lymphocyte proliferation. Furthermore, potential effects on the function of the immune system were measured by the T cell-dependent antibody response (TDAR) to KLH and the delayed type hypersensitivity (DTH) response upon intradermal KLH injection. 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 in Göttingen Minipigs®.

The oral dosing of the immunosuppressive compounds was chosen at such dose levels that no clinical signs were observed. Body weights were only affected in the Dexamethasone treated animals and showed a tendency to lower body weights in male and female animals from day 21 onwards (only significant for females). From the results obtained, it was clear that the different assays showed variable sensitivity in assessing immunosuppressive effects of Cyclosporin A and Dexamethasone. Cyclosporin A had a clear effect on DTH responses and the KLH specific antibody responses. Dexamethasone inhibited ex vivo proliferative responses of PBMC (in female minipigs only), NK activity of PBMC, and the IgM and IgG antibody responses to KLH (the latter being less pronounced than in Cyclosporin A treated animals).

At necropsy, macroscopically no deviations were observed other than a reduction in thymus size in Dexamethasone treated animals (see figure 1). The absolute and relative thymus weights of both the Cyclosporin A and Dexamethasone treated animals were reduced, which was only significant for the Dexamethasone treated minipigs. Histopathology of the thymus revealed the well known distinct reduction in the cortex : medulla ratio. Spleen weights and histology were not affected.

From the results of this study it is clear that the potential immunotoxicity of pharmaceuticals can be assessed in Göttingen Minipigs®. However, as considerable variation between animals was observed, larger numbers of animals will be necessary for sufficiently high power to determine immunosuppressive features of new substances. Furthermore, it is advisable to include multiple endpoints in a study, as we observed the differential effect of two known immunosuppressors on different read-out parameters.

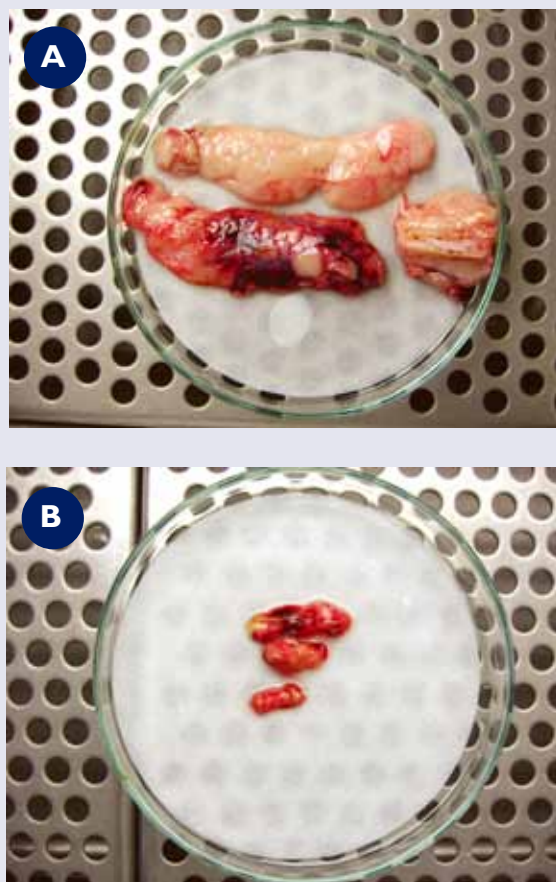


Figure 1: Representative thymuses of A) a vehicle control and B) a Dexamethasone treated female minipig

AUXILIARY EQUIPMENT – NEW BITE BAR

We have learned from one of our customers that they have successfully used a different type of bite bar in conjunction with oral dosing. This device allows the technician restraining the pig to open the pig's mouth and control the movements of its head at the same time. This enables the other technician to use both hands to administer the gavage or a capsule.

The bite bar is available from Ellegaard, and if you would like a demonstration, please sign up for one of our Handling and Dosing courses.

Bite bar



Restraint bench for blood sampling



Restraint chair for gavaging

Sling frame



Minipig scale

SURGERY AND ANAESTHESIA COURSE PLAN 2009

So far two courses have been planned for the rest of 2009

- 10 – 11 June
- 9 – 10 September

but additional courses may be arranged if at least 3 persons want to register and it can fit into the instructor's plans. 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.dk and learn more.

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Thank you

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NEW ARTICLES ABOUT MINIPIGS

- *A novel spatial Delayed Non-Match to Sample (DNMS) task in the Göttingen minipig.*
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- *The adult Göttingen minipig as a model for chronic heart failure after myocardial infarction: focus on cardiovascular imaging and regenerative therapies.*
Schuleri KH, Boyle AJ, Centola M, Amado LC, Evers R, Zimmet JM, Evers KS, Ostbye KM, Scorpio DG, Hare JM, Lardo AC.
Comp Med. 2008 58(6): 568-79.
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Jung M, Breusch S, Daecke W, Gotterbarm T.
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- *Vascular endothelial growth factor-165 gene therapy promotes cardiomyogenesis in reperfused myocardial infarction.*
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ELLEGAARD GÖTTINGEN MINIPIGS DVDs AVAILABLE

The Göttingen Minipig – Handling and Dosing

This DVD is available in a German language version as well as in French and English. It is 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 do not hesitate to contact us for further information at ellegaard@minipigs.dk or phone +45 5818 5818.

