Ellegaard
GÖTTINGEN MINIPIGS A/S

Clean pigs for clear results

Newsletter 37

Spring 2012

POSTER:
Toys – Enrichment of Göttingen Minipigs

Attend a Minipig Continuing Education session!

How to handle and diagnose
clinical signs in minipigs!

Highlights from the 2011 meeting of the
Minipig Research Forum

Join the Minipig Research Forum Group on LinkedIn!

POSTER:
Early mating of young Göttingen Minipigs

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Soroe Landevej 302 · DK-4261 Dalmose · Denmark · Tel. +45 5818 5818 · Fax +45 5818 5880 · ellegaard@minipigs.dk · www.minipigs.dk
For Göttingen Minipigs in North America please contact Marshall BioResources – infous@marshallbio.com
For Göttingen Minipigs in Japan please contact Oriental Yeast Co., Ltd. - fbi@oyc.co.jp

DEAR READER

Although 2012 had very cold beginnings, we are now enjoying the first signs of spring. The days are getting longer and, with this in mind, I would like to shed light on some of last year's highlights and achievements.

First of all, we sold more minipigs in 2011 than ever before. This is very positive because it confirms that the Göttingen Minipig has proved to be as suitable for many types of studies as was originally expected when the company was founded. Even so, it is important for me to emphasize that we do not sell minipigs at any price. It is our aim that a thorough and justified species selection will be performed before every study so that the minipig is chosen for studies where it is the most suitable model.

In this connection, we are deeply satisfied that knowledge about the minipig is constantly being elaborated upon. The sequencing of the minipig genome has been of great interest to us, and to the entire industry. It has made it possible to make more specific comparisons between minipigs and other species.

The publication of the book "The Minipig in Biomedical Research" was another highlight of 2011. The book provides readers with useful information about the minipig as an animal model and I highly recommend it.

In 2012, we would like even more information about minipigs to be developed, collected and shared. We will prepare Minipig Continuing Education (MCE) sessions in major cities in Europe where the minipig will play the leading role. Minipig experts and consultants will serve as supporting characters to update new and existing minipig users about this specific species. More information about these MCE sessions is available in this newsletter.

Our contract with the Georg-August University of Göttingen has been renewed, which means that we continue our rewarding collaboration for another 15 years. The University continues its global management of the Göttingen Minipig genetics and provides us with information that enables us to select the best breeders and optimise our breeding efforts.

Our co-operation with Marshall BioResources and Oriental Yeast Co. is certainly also worth mentioning. Marshall is doing an excellent job supporting minipig customers in North America. In Japan, Oriental Yeast Co. has finalized the building of a new minipig breeding facility so that a breeding herd can soon be transferred to them to supply the Japanese market with Göttingen Minipigs.

While helping Oriental Yeast Co. to build their minipig facility, we continuously focus on how to improve our own facility in Denmark. In 2012, the heating of our facility will be made carbon neutral when we start using woodchips for heating instead of oil. We look forward to this and other improvements that will follow in the years ahead.

Lars Ellegaard, who imported the first Göttingen Minipig breeders, will retire in April 2012. Words cannot express how much Lars means to this company, and we all appreciate his entrepreneurship and hard work over the years. I wish him all the best and I am sure he will enjoy his retirement. We foresee an interesting and successful future for the Göttingen Minipig and we have a strong team that will continue when Lars retires.

In this newsletter, our veterinarian, Helle Lorentsen, gives you an overview of how to handle and diagnose clinical signs in minipigs. We are in contact with many minipig users and, when dealing with health issues, it is valuable for us to have all the information pertaining to the specific incident.

A poster about enrichment for Minipigs and a poster from a project about the early mating of Minipigs are included in the newsletter. The newsletter also includes further details about the MCE sessions, a Minipig Research Forum Group on LinkedIn and the 2011 meeting of the Minipig Research Forum.

Sincerely,
Jens Ellegaard
CEO, Ellegaard Göttingen Minipigs A/S

CONTENT

RETIREMENT

In 1982, Lars Ellegaard imported the first Göttingen Minipigs to Denmark. A lot has happened since then: over the years Lars was deeply and actively involved in improving the health status of the Göttingen Minipig, disseminating knowledge about it and supporting minipig users all over Europe – even overseas as well.

On 1 April 2012 Lars will retire and we would like to thank him for paving the way for the Göttingen Minipig in the pharmaceutical industry. He has cooperated with many people to make the company what it is today: a global breeder and distributor of not only the Göttingen Minipigs themselves but also of the knowledge and support/service relating to them.

We have found some old photos we would like to share with you and some new photos showing the kind of work Lars will be doing from now on. His fishing boat will definitely be getting more attention in the future.

Lars at one of his numerous trade shows (1990's)

Breaking ground for the first minipig barrier facility (1992)

Lars delivering Minipigs in his van (~1985)

After his retirement, Lars will spend much time on this fishing boat

How to handle and diagnose clinical signs in minipigs

This letter is specifically addressed to those of you who sometimes contact Ellegaard with questions about clinical signs you have observed. A checklist will be introduced.

As a veterinarian at Ellegaard, I am often asked for advice and guidance when minipigs show clinical signs before, during or after a study. I am very pleased to be informed by the findings and to maintain good dialogue with you.

I believe that it will be in everyone's interest and to everyone's benefit – scientist, study director, you and me – if the information in these cases is as complete as possible with respect to facilitating a tentative or conclusive diagnosis.

Therefore, I have designed a checklist which can help you to consider different aspects, including housing conditions and epidemiological reflections, and remind you to do your clinical and diagnostic homework before the animal is euthanized or dies spontaneously.

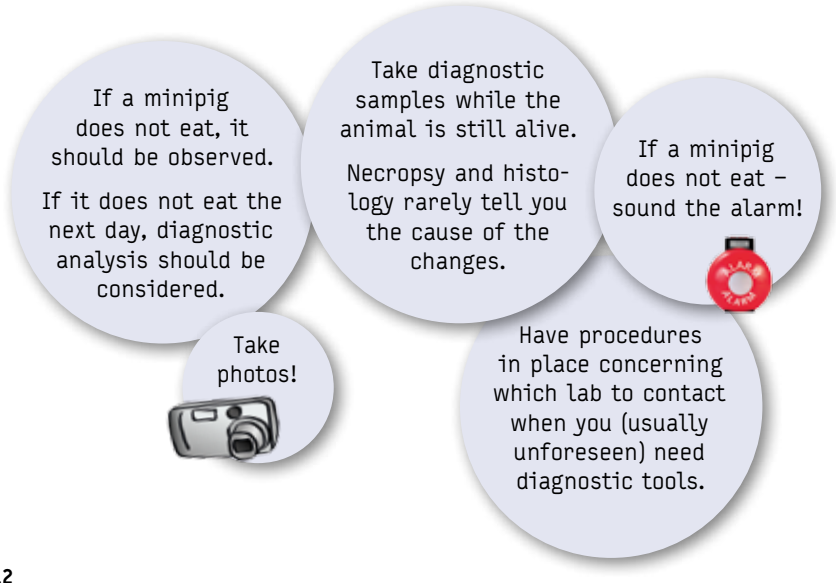
The extracts of the checklist shown on this page may give you an idea of the kind of information that I suggest is valuable. The entire "Clinical findings" scheme can be found on www.minipigs.dk and will also soon be distributed to you in an e-mail.

When you contact Ellegaard for advice in these matters, I hope you take the information in the checklist into consideration. It may be the kickoff to dialogue which hopefully leads to a diagnosis.

For further information please contact Helle Lorentsen, DVM, hl@minipigs.dk.

CLINICAL OBSERVATIONS	
Minipig ID (no., gender, date of birth)	
Date of delivery and onset of study	
Conventional swine or other species in the animal unit – describe	
Description of acclimatization/ housing - preventive medication - group/single housing - socialization programme	
Other affected animals - ID, age, symptoms, analyses	

ANALYSES AND RESULTS	
Necropsy/gross pathology	
Microbiology (e.g. swabs), date of sampling	
Serology, date of sampling	
Clinical hematology, date of sampling	
Hematology, date of sampling	
Histopathology	
Further analysis planned	
Your tentative diagnosis	
Photos or other documentation:	



Minipig Continuing Education sessions

October 17 and November 28 2012 we will arrange Minipig Continuing Education (MCE) sessions in Paris and Frankfurt respectively. These sessions will provide an in-depth coverage of four key areas in safety assessment of pharmaceuticals: *ADME/DMPK, Toxicology, Animal Welfare* and *Pathology*. A fundamental understanding of the minipig and how it compares to the non-human primate, the dog and ultimately the man is necessary in order to ensure that the most relevant non-rodent species is selected.

Target audience: Professionals from contract research organisations (CROs) and pharmaceutical companies working with ADME/DMPK, toxicology and pathology who wish to expand their knowledge about minipigs in these areas. Attendees will improve their skills and competencies, which will be beneficial for them as well as for their organisations.

ADME/DMPK studies in minipigs
Lars Dalgaard, Independent Consultant, France

This talk will compare the ADME properties of species used in toxicity testing of drugs in development with focus on minipig and the enzymes responsible for metabolism. The impact of regulatory requirements on ADME studies, including species selection and metabolism in toxicity testing, is discussed. Considerations concerning molecular structures in relation to the choice of species are presented. What should be done to improve the selection of species? When is the use of minipigs as first choice warranted?

- Species comparison in drug metabolism – where is the minipig compared to dog, non-human primates, and man?
- Cytochrome P450 (microsomal enzymes)
- Aldehyde oxidase (cytosolic enzymes)
- Conjugation reactions (both microsomal & cytosolic enzymes)
- The importance of transporters for the availability of drug at the intended site of action.
- Case studies

Minipigs in Non-clinical Drug Safety Studies (Toxicology & Safety Pharmacology)
Peter Clausing, Independent Consultant, Germany

In the past, dogs were routinely used without considering their scientific value on a case-by-case basis. Since several years international guidelines (e.g. CPMP/SWP/1042/99) require that species for drug safety assessments be selected according to their similarity to humans. In addition, the new European animal welfare legislation (Directive/2010/63/EU) aims at reducing the use of non-human primates in the long run, making the search for alternative non-rodent species even more urgent. Meanwhile the minipig is not only one of the best established alternatives, but it is also a species with many similarities to humans. The presentation will cover:

- Species specific drug class effects (comparing minipigs with other species)
- Species specific properties (comparing minipigs with other species and humans)
- Practicalities of using minipigs in drug safety studies, including inhalation and reproductive studies

Animal welfare of the Göttingen Minipig
Helle Lorentsen, Head of Veterinary Services, Denmark

- Housing and husbandry
- Enrichment - physical and social
- Behaviour
- Socialisation and training
- Handling and dosing procedures

General knowledge about the minipigs is important in order to meet their specific needs.
When the basic needs of the minipigs are in place, better animal welfare is not necessarily a question of economic investments.
Sometimes even small changes in daily routines can result in high quality enrichment of the minipig.
This will be elaborated in the presentation and illustrated by video recordings of the minipigs in action!

Pathology of minipigs
Roger Burnett, Independent Consultant Toxicological Pathologist, United Kingdom

The aim of the talk is to outline the various challenges that are presented by the minipig for the necropsy team, the histologists and the pathologists and how to overcome them. The anatomical peculiarities of the minipig will be discussed and how they modify the necropsy procedure. Tissue sampling and preparation will be discussed with consideration of histological preparation. There will be an introduction to the basic histology of the minipig highlighting differences between them and other laboratory species. The major lesions seen as spontaneous background in the minipig will be discussed in respect of their frequency and severity. Particular emphasis will be placed on the interpretation of these lesions in toxicological studies and how the range of lesions compares with those seen in the beagle and non human primates. The influence of the pathological background of the minipig and that of other large laboratory animals will be discussed in relationship to various drug classes, giving the pathologist's perspective on species selection.

The MCE sessions will begin at 9:00 and end at 15:00. If registering for a session before June 1 the registration fee is €100. After June 1 the fee is €200. Coffee, refreshments and lunch are included.

Please contact us to receive further information about these sessions or to register.

Highlights from the 5th Minipig Research Forum

Peter Clausing
Karina Riget Nielsen

On last year's 28-29 November, the 5th Minipig Research Forum (MRF) took place in Frankfurt, a city easy to reach from various parts of Europe and the rest of the world. This and the interesting scientific program contributed to the record number of more than 70 participants, including such from Japan, China and the U.S. The 19 presentations, all of high quality, and a couple of posters offered one and a half days packed with new information and interesting discussions. Due to local circumstances, the posters unfortunately did not gain as much attention as they deserved – something to change in the future. The presentations focused on various aspects of developmental toxicity, including juvenile studies and reviews of the pre- and postnatal development of selected organ system, neurobehavioral testing and a number of disease models in minipigs.

P. Hyttel started out with an interesting overview of the minipig reproductive biology in comparison with the reproductive systems of humans, dogs and non-human primates which was of great interest to the participants.

T. Andreassen from CiToxLAB in Denmark offered two presentations. The first was an overview of the scientific considerations and practical execution of embryofoetal developmental toxicity studies, and the second was an introduction to a project to create a more sophisticated database on congenital abnormalities of Göttingen Minipigs (note: data of such type was collected and described in the past – see Damm-Jørgensen, 1998: Scand. J Lab Anim. Sci., Vol. 25, Suppl. 1. 63-75).

An important topic concerning the conduct of embryofetal (Segment II) studies in minipigs was adressed by **E. Marsden** who talked about using pre-mated sows and early termination (on gestational day 60 instead of 110) in such type of studies. This approach is not undisputed, but has the potential to save capacity. Marsden showed convincing data of a study aimed at the validation of this approach.

Although it is generally known that the (mini)pig's gastrointestinal system is quite similar to that of humans details are often lacking. Thus, **C. van Ginneken**'s up-to-date review which included developmental aspects and comparisons to other offered some difficult to find information and attracted significant attention.

Thanks to **R.W. Thon**, participants of this year's MRF are now well informed about the availability of transgenic Göttingen minipig models for atherosclerosis (model in use), psoriasis (partially usable) and Alzheimer's disease (no phenotype yet). According to the author, a lot has been learned during the establishment of these transgenics, i.e. there is more to come.

An embryo-foetal developmental study of a peptide with a MW approximately 2000, using minipigs was the topic of the presentation by **I. Brück Bøgh**. This was one of the first studies were the minipig was employed as a non-rodent species for safety evaluations of biologics – a domain previously reserved for non-human primates.

With his presentation **N. Downes** from Sequani provided the participants with an overview of the histology of the reproductive organs. Such information is very important when considering the suitable age of animals for reproductive toxicity studies.

P. Howroyd and co-authors gave a comprehensive overview of the peri-pubertal phase of development of female Göttingen minipigs, based on re-reading histological slides of 39 control group animals. The take-home message was that based on their definition and results the early sexual maturity of minipigs (as compared to dogs) warrants further investigations, which have been initiated.

A. Penninks presented information about the developing immune system of minipigs and the results from studies performed at TNO Triskelion.

S. van Cruchten offered a glimpse at the important work ongoing in the area of intestinal absorption and metabolism. In particular the ontogeny of the P-glycoprotein carrier and CYP3A was discussed. The question whether P-glycoprotein is just as an important transporter in pigs as in humans (although likely) will be addressed in the future.

P. Clausing's otherwise rather comprehensive review of neurobehavioral testing in minipigs was lacking one important aspect – testing of spatial memory which will become even more important as soon as a minipig model for Alzheimer's disease is available. Fortunately this gap was covered by no less than 3 presentations, including that of **N. Grand** who gave an overview of her experience with juvenile studies, including learning tests, and both **E. Gieling** and **J. Manton** who successfully employed the hole-board for testing spatial learning and memory.

P. Stott actively participated in studies involving bottle feeding of minipigs. She shared her successful experiences with bottle raising of minipigs as a useful technique for juvenile studies of a certain study design.

S. Maguire gave insight into the current status of proposed vs. required juvenile studies in Pediatric Investigations Plans submitted to the EMEA with special reference to the minipig. An important topic was covered by **L. Dalgaard** who presented an overview of the maturation and development of metabolism in minipigs.

Last, but not least, **H. Lorentsen's** lively presentation on environmental (and social) enrichment used at the Ellegaard breeding facilities was a successful "poster-converted-to-a-talk" on a very short notice due to the cancellation of another presentation. All who have missed this year's MRF should mark their calendar for 26-27 November 2012, when the next MRF will take place - again in Frankfurt, Germany.

Educational package

We continuously develop and collect knowledge about minipigs and we would like to share this knowledge with you. In collaboration with experienced, independent consultants we have prepared an educational package that consists of the following topics:

- Minipigs as a non-clinical model in ADME studies for the development of drugs
- Minipigs in toxicology studies
- Immune system of pigs and minipigs
- Animal welfare of the Göttingen Minipig
- Minipig welfare - Acclimatisation and socialisation
- Handling, dosing and training of the Göttingen Minipig
- Cardiovascular safety pharmacology in the minipig
- Implantation of a central venous catheter in Göttingen Minipigs
- The histology of the Göttingen Minipig

The content of the different parts of the educational package is described in the following abstracts.

Please contact us (ellegaard@minipigs.dk) if you would like to receive parts of the educational package.

The educational package will be updated continuously and you are always welcome to contact us if you would like an updated version.

Minipigs as a Non-clinical Model in ADME Studies for the Development of Drugs

Lars Dalgaard

This document aims to compare the ADME properties of species used in toxicity testing of drugs in development by focusing on the minipig and the enzymes responsible for metabolism.

The impact of regulatory requirements on ADME studies, including species selection and metabolism in toxicity testing, is discussed. These requirements include the FDA, MIST guideline, the MIST consensus paper, the ICH M3 guideline, and the draft DDI EMA guideline.

The timing of studies to give the optimal development plan is outlined. This includes the merged mass balance and metabolite profiling studies, bioanalytical strategy – the tier approach, early metabolite identification using hyphenated chromatographic-spectroscopic techniques, and in vitro testing.

Based on the putative metabolism of specific molecular structures, a qualified opinion of the choice of species can sometimes be made.

To improve the selection of species, in vitro testing using microsomes, cytosol, S9, and hepatocyte from rodents, minipigs, dogs, and primates should be compared. Based on the results, it is possible to optimise the choice of the right species.

The use of minipigs as a non-rodent species in the development of a new drug in non-clinical safety studies including ADME and toxicity studies is warranted in many cases. Minipigs are the first non-primate choice when the drug candidate is metabolised by aldehyde oxidase that is practically non-existent in dogs, but present in pigs. This also applies to N-acetyltransferase substrates (NAT1 and NAT2) that are practically absent in the dog liver. Finally, CYP2C9 substrates might also give rise to concerns about using dogs as a non-clinical model.

Minipigs in Toxicology Studies

Peter Clausing

In a standard package of non-clinical drug safety studies, regulatory authorities require the use of a rodent and a non-rodent species. The typical species employed have been:

- *mice*: for single and sometimes repeat dose studies, and carcinogenicity studies;
- *rats*: used for single and repeat dose studies, for reproductive studies, and carcinogenicity studies;
- *rabbits*: for dermal studies, local tolerance studies, and for reproductive studies;
- *dogs*: for single (Japan only) and repeat dose studies; and
- *nonhuman primates*: if dogs were not suitable.

In the past, the RAT/DOG approach was seldom questioned, and it was rarely scrutinised whether this species combination was appropriate for the particular project. This has now become a regulatory issue and, when decisions are made concerning the choice of species, it is now a requirement to provide a rationale to the authorities for the species selected.

In the past, pigs and/or minipigs were rarely even considered for use as the non-rodent species of choice.

More recently, the particular value of the minipig has been identified as the non-rodent species generally suitable for many of the toxicity studies employed during the development and safety assessment of pharmaceuticals.

The purpose of this educational package is to explain the reasons

- why many pharmaceutical companies have begun to include minipigs in their selection of non-rodent species in drug safety studies; and
- that regulatory authorities worldwide have begun to accept minipig studies in submissions.

Immune system of pigs and minipigs

Niels-Christian Ganderup

This text contains an overview of the immune system and immune function of the pig as presented in Bode et al. (2010) as well as information on the use of the Göttingen Minipig in immunotoxicity, immunogenicity testing and juvenile immuno development.

Pigs have a well characterised immune system and have been subject to extensive research in this area for decades. Published literature pertains predominantly to normal/farm pigs; less information specifically pertaining to minipigs is available.

It is important to stress that there is no obvious reason to believe that significant differences between normal/farm pigs and minipigs exist, however, ad hoc investigations bridging immunological information from minipigs and normal/farm pigs are always advisable.

Animal Welfare of the Göttingen Minipig

Helle Lorentsen

This document is a short introduction to the overall considerations on how to pursue the best welfare of the minipigs. Specific guidelines are given on how to implement and maintain good minipig welfare in every aspect.

A high degree of animal welfare is valuable to both animals and humans and this is imperative in performing biomedical research of high scientific value.

It is worth remembering that animal welfare is not only about adjusting a few welfare parameters. It is about taking every single parameter into consideration and to optimize wherever possible. The result is an additive effect.

Ensuring a high degree of animal welfare can result in more reliable study results.

Good animal welfare is a matter of being able to turn different welfare buttons and continuously adjust in order to provide the animals with optimal conditions.

Acclimatisation and socialisation

Helle Lorentsen

This text focuses on the time after the minipig has been transported from the breeder until the start of the experimental procedures. Apart from general considerations regarding a beneficial acclimatisation period, specific instructions – including suggestions for a socialisation programme – are provided.

Many people working with minipigs are already familiar with most of the content of this text. However, the point is that sometimes it is worth looking at the routines, reconsidering some aspects and discussing whether there is room for improvement. And realising that it is not all or nothing, but rather that small improvements and differences can have a positive effect on the welfare of the minipigs.

The overall purpose is to improve general minipig welfare and conditions during the acclimatisation period. By ensuring good minipig welfare and conditions the animals will be easier to handle during experiments and this will ultimately contribute to valid study results.

Once and for all, it is important to emphasise that acclimatisation is not a matter of leaving minipigs in peace without any disturbance. On the contrary, acclimatisation involves gradually accustoming the animals to experimental conditions in a way that takes account of animal wellbeing.

Handling, Dosing and Training of the Göttingen Minipig

Adrian Zeltner

For any type of work with animals, a profound understanding of the species involved is paramount. Conducting studies with Göttingen Minipigs in a research environment is no exception. Proper handling and training of the minipig will greatly contribute to the success of the study. In lack of scientific data, it is assumed that the basic ethology of the Göttingen Minipig is no different from other porcine strains, either wild or in captivity. Practical experience backs up this assumption, but relevant research in this field is needed, especially concerning the extent to which selective breeding may have modified the needs and behaviour of the Göttingen Minipig.

People with experience working with dogs can still apply their basic animal interaction skills but should be aware that dealing with a minipig requires a different approach altogether. As dogs have been bred for human companionship for thousands of years, understanding their behaviour almost comes naturally to us. Pigs have been domesticated for many years as well, but only as a source of food, despite the fact that pigs can be excellent companions. Our attitude towards them, and vice versa, is therefore slightly different. Another important difference in respect to the dog is that pigs are not predators, but prey. This means that we are dealing with a wary and shy animal. It takes some effort to gain its trust, but once established Göttingen Minipigs can be accustomed and trained to actively participate in a study. This creates a situation where humans work with the minipig rather than against it, thus drastically reducing the stress factor for everyone involved.

Cardiovascular Safety Pharmacology in the Minipig

Michael Markert

The use of pigs and minipigs for biomedical research has demonstrated that they offer potential advantages when certain comparisons to humans are desirable. For instance, the minipig has a similar heart-to-body weight ratio and a coronary artery distribution similar to humans. Moreover, cardiac anatomy, metabolism and electrophysiology in pigs are comparable to humans. Initial analyses show that the major myocardial ion currents responsible for the human myocardial action potential are also present in the Göttingen Minipig. At the same time, the use of the dog as an experimental animal has drawn criticism due to its role as a companion animal. In contrast, the pig is still viewed primarily as a farm animal with fewer emotional implications.

ECG data from minipigs are available from animals restrained in a sling with external limb leads. Data for left ventricular pressure from unrestrained minipigs has only been published recently. LVP measurements are particularly useful in that the derivative of LVP, i.e. LVdP/dt, is a well-established parameter for the assessment of cardiac contractility.

Drug-induced prolongation of the QT interval (a marker for a delay in cardiac repolarisation) of the electrocardiogram (ECG) has drawn increasing attention from regulatory agencies and the pharmaceutical industry, and detecting these effects has had a great impact on drug discovery and development. The delayed repolarisation, frequently attributable to blockade of the rapidly activating delayed rectifier potassium channel, IKr, favours the genesis of early afterdepolarisation (EAD), which can initiate arrhythmia. Additionally, the prolongation of the QT interval by drugs is often associated with increased heterogeneity of cardiac repolarisation, a substrate for a re-entrant mechanism responsible for sustained arrhythmia, and preclinical testing for tolerability and safety is required in both rodents (usually the rat) and in a second, non-rodent species. Nevertheless, there are some 'safe' drugs that inhibit IKr and cause QT prolongation without inducing TdP. In any case, it is desirable that models used for testing new drugs for potential effects on the QT interval are shown to have the sensitivity required for detecting subtle changes. A known hERG-blocking agent, Moxifloxacin, as well as a ß-adrenoceptor blocker, Propranolol, have been tested in this study. Moxifloxacin has been recommended as a positive control for clinical trials assessing QT prolongation potential as it produces a consistent, highly reproducible effect on QT interval duration. Propranolol is a non-selective ß-adrenoceptor blocker mainly used in the treatment of hypertension. It is expected to decrease heart rate and reduce myocardial contractility. Both compounds had the expected effects on the Göttingen minipig. These, and other data, support the Göttingen minipig as a sensitive cardiovascular and electrocardiographic model for safety pharmacological evaluation of new pharmaceutical agents.

Implantation of a Central Venous Catheter in Göttingen Minipigs

Adrian Zeltner

The main reason for using a CVC in a minipig is to facilitate multiple blood sampling. It reduces the stress on the animal, improves the welfare and decreases the number of employees required for the procedure.

Although this method entails some risks and challenges, if properly managed it can be successfully executed after a minimum of training.

The purpose of this paper is to inform about the material and methods tested at Ellegaard Göttingen Minipigs. They are subjective and by no means exhaustive.

A central venous catheter is defined as a catheter whose tip remains in the central circulatory system. There are various types of these catheters, but, for the purpose of this guide, we will focus on short-term (< 30 days) IV access and percutaneously-inserted catheters. These catheters are marketed by various manufacturers. There are single, double or multi-lumen catheters on the market, almost all made of PU and available in a variety of diameters. We have chosen to work with large-diameter single lumen catheters to minimise the risk of blockage due to thrombosis.

The use of a dual lumen catheter, one lumen for dosing and the other for sampling, involves a risk of contaminating the blood sample with a compound as the two distal ports are close to each other. On the other hand using two lumens for sampling could provide an alternative if one gets blocked. This study has not tested the veracity of these assertions.

Vena jugularis ext./vena cava cran. is the site of catheterisation because these vessels are large, even in small minipigs. This site poses some challenges due to the anatomy of the pig, however.

Catheterisation should be executed using aseptic procedures. It is done under general anaesthesia, using the Seldinger technique or a modified Seldinger technique, the day before sampling. Catheter patency can range from 3 to 28 days, depending on a multitude of factors.

The use of a needle-free IV connection device (Bionector™) proved very useful.

Histology of the Göttingen Minipig

Roger Burnett

These notes have been produced to accompany the double DVD set on the Göttingen Minipig Histology. The DVD contains scans of the original histology control slides of sexually mature minipigs from MDS. The Aperio scan with "Imagescope©" program gives a "virtual slide" which allows navigation around the tissues and magnification, just as if the tissue was viewed through a microscope, allowing an examination of the images with up to x 20 magnification.

The notes have been prepared to help those not used to looking at tissues to an understanding of the images on the DVD's and the differences in the minipig. This is a quick start guide and has no pretences to be a "text book" but an attempt to bring information from various sources together in order to introduce newcomers to the minipig.

The majority of the articles referred to in the text are on the CD, "The Göttingen Minipig Articles and References".

All the figures have been captured from the scanned sections using the "Imagescope" program, so the same areas can be found in the scan. The magnifications given with the figures are the equivalent objective magnification. In a few cases a further photographic enlargement has been made to highlight a particular feature. The mean organ weights from some 20 pigs per sex are given for each tissue at 8 weeks and 6 months of age to give some idea of the size and growth. The full data set with SD and ranges is presented in Appendix 1.

The text for each tissue or group of tissues has been laid out to allow easy updates to this rapidly changing data.
In general, histology of the minipig is very similar to that of other species, the notable difference being the inside-out lymph nodes. The anatomy has a few twists, the thyroid is a single entity and the parathyroids are not easy to find. The pathology is remarkably clean and only a very few background changes are seen.

The Minipig in Biomedical Research

is a comprehensive resource for research scientists on current and future use of the minipig in basic and applied biomedical research and the development of drugs and chemicals. Written by acknowledged experts in the field, and drawing on the authors' global contacts and experience with regulatory authorities and the pharmaceutical and other industries, this accessible manual ranges widely over the biology and scientific and practical uses of the minipig in the laboratory. Its coverage extends from the minipig's origins, anatomy, genetics, immunology and physiology to welfare, health, and husbandry; practical dosing and examination procedures; surgical techniques; and all areas of toxicity testing and the uses of the minipig as a disease model. Regulatory aspects of its use are considered.

Readers will find an extensive amount of theoretical and practical information in the pharmacology, ADME and toxicology chapters which will help scientists and managers when deciding which species to use in basic research, drug discovery, and pharmacology and toxicology studies of chemicals, and biotechnology products and devices.

The book discusses regulatory uses of minipigs in the evaluation of human and veterinary pharmaceuticals, medical devices, and other classes of xenobiotics. It describes features of normal health, normal laboratory values, and common diseases. It also carefully elucidates ethical and legal considerations in their supply, housing, and transport. The result is an all-inclusive and up to date manual about the experimental uses of the minipig that describes 'How to' and 'Why' and 'What to expect in the normal', combining enthusiasm and experience with critical assessment of its values and potential problems.

Contents

Origin and Management of the Minipig
History and Development of Miniature, Micro- and Minipigs
Friederike Köhn (DE)
Husbandry and Management
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Early mating of young Göttingen Minipigs

Helle Lorentsen, DVM; Lea Bruun, animal technician; Mads Thorling, animal technician; Ellegaard Göttingen Minipigs A/S

24 minipigs

	Number of minipigs	Percentage
No detection of heat	15	63 %
Detection of heat	9	38 %
Successful mating at the age of 5 ½ months in average	8	33,3 %
Gestancy	5	21 %
Farrowing	5	21 %

Background

According to literature the female Göttingen Minipig is sexually mature approximately at the age of 4-5 months.
At Ellegaard Göttingen Minipigs in Denmark, female minipigs selected for breeding are mated at the earliest when they are 7½ - 9 months old. For experimental purposes a low bodyweight at the first time of mating can be desirable. Therefore younger minipigs (5-6 months) have been mated in order to evaluate the success rate of mating, fertilization, gestation and finally parturition.
The issue has been debated in the local animal welfare committee. It was pointed out that the mating should take place with boars of equal size and that the wellbeing of the pregnant minipigs should be carefully monitored during parturition and the peri- and postnatal period.

Detection of heat

24 minipigs between 3½ and 4½ month (9.4 – 13.8 kg) were in a period of 7 weeks observed for clinical signs of heat: swelling and reddening of the vulva and tendency to mount the other minipigs.
To promote visual signs of heat, a boar was walking in the corridor approximately 5-10 minutes every day.
Of the 24 animals, 3 animals showed visual signs of previous heat.

Mating and gestation

8 minipigs were successfully mated and they were gathered in a pen.
Gestation tests were performed with an ultrasound scanner 4 and 6 weeks after mating. Gestation shows as visual signs of embryonic vesicles.
5 minipigs were tested positive for gestation at 4 and 6 weeks after mating. 3 minipigs were tested negative at both gestation tests.
1 week before expected parturition the gilts were moved to the farrowing section and housed individually.
The gestation period of all 5 gilts passed without irregularities.

Farrowing

In average, farrowing started 1.5 days before expected.
All farrowings took place with no irregularities.
Litter sizes ranged from 3 – 5 piglets (in average 4.2 piglets). Older sows normally have bigger litters than gilts.
Weight of piglets ranged from 200g – 500g (average 400 g). Average size of newborn minipigs in general is around 500 g.
After birth, piglets were added to the gilts for cross-fostering and both sows and piglets were weaned in good body condition.

Conclusion and discussion

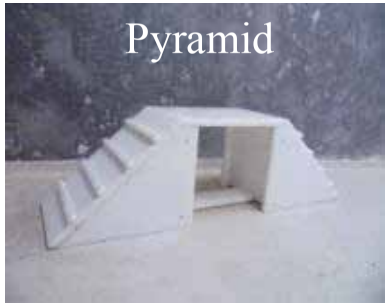
It took 24 minipigs to obtain 5 pregnancies (pregnancy rate 21%).
It is shown that it is physiologically possible and, from an animal welfare point of view, safe to mate female minipigs at a young age. As it is difficult visually to spot female minipigs in their first heat, the second heat for mating seems to be more convenient from a practical point of view.
Ellegaard has previously mated young female minipigs after heat synchronizing. The pregnancy rates were up to 80% for animals mated at 6 months of age.
Further investigations are needed to learn more.

Toys – enrichment of Göttingen Minipigs

Lorentsen, H., DVM, Ellegaard Göttingen Minipigs
Nielsen, P., Animal Technician, Ellegaard Göttingen Minipigs

European Directive (2010/63/EU): *…any restrictions on the extent to which an animal can satisfy its physiological and ethological needs are kept to a minimum;*
Therefore different types of enrichment should be offered to minipigs in order to make them able to express normal behavior and avoid boredom.

Minipigs are curious and explorative by nature



Anchor the toys to avoid contact with manure

Observation: Bucket in chain

Groups	Toys	Observation time		
		10:30	11:30	14:30
		Score of interest ☹ 1 – 5 ☺		
female	on/off*	5	2	1
male	on/off*	4	2	1
female	ad libitum**	2	1	1
male	ad libitum**	2	1	1

* Toys are given 10:30 a.m. and removed 14:30 p.m.

Method

5 different types of toys have been assigned in order to compare the popularity of the toys among the minipigs.
The minipigs, aged 3-5 months, were housed in groups ranging from 10-15 animals.
Each toy was tested one week among 4 different groups of minipigs.

Toys can´t replace bedding and rooting material. Bedding and rooting material

** Toys are in the pen 24/7

should always be available

Comparison of popularity of toys (on/off group)

High fun factor:
***biting**
***manipulation**
***exploring**

	Score ☹ ☺ 1 - 5	Comments
Plate with bite stick	3	Fun for a short time Ends in the manure
Bucket in chain	5	Great fun! Many different functions: pushing, biting, hiding, mounting
Metal chains	3	Not play – more satisfying the needs for biting
Pyramid	5	Climbing on top and seeing the world from a new angle!
Ball in chain (with food pellets)	4	Great fun as long as there are food pellets in it!




Toys should be changed and replaced on a daily basis

Conclusion

The results show that the interest in the toys is significantly higher among the minipigs with on/off-toys than among the minipigs with unlimited access to toys.

The minipigs get bored with toys as soon as novelty is gone. This is significantly illustrated in the table above – the interest decreases even after 1 hour.

Therefore it is crucial for the quality of enrichment that the **toys are changed and replaced with new ones on a daily basis**.

Try to make the day as exciting as possible: feeding, provision of bedding material, provision of toys should be at different times.

Bedding- and rooting material should always be available.

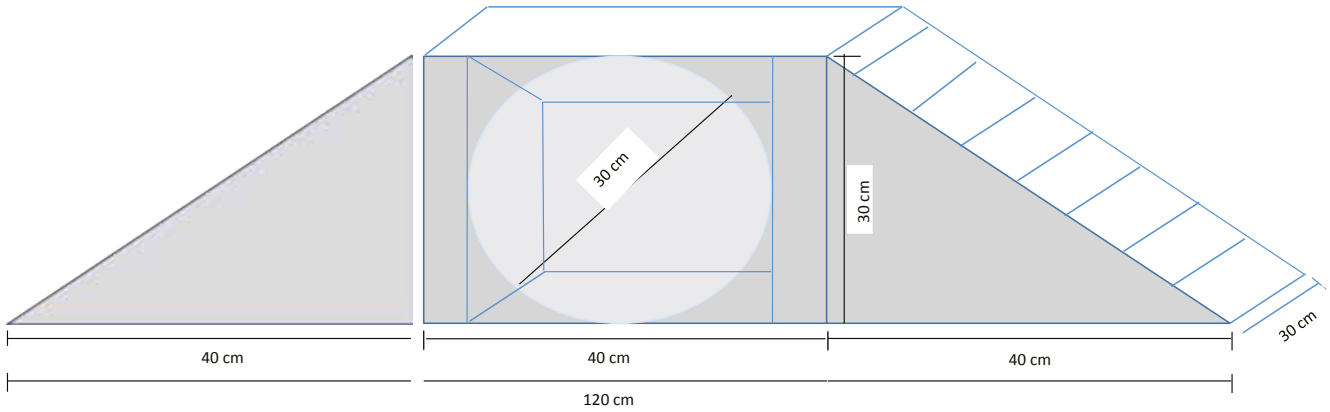
Current observations in the barriers at Ellegaard show that single-housed minipigs pay less attention to toys than group-housed minipigs.

Female minipigs seem to show significant more interest in toys than male minipigs.

Elder minipigs tend to show less interest in toys than young ones.

If the toys get soiled by the manure the interest decreases almost momentarily.

A little walkabout outside the pen is high quality enrichment for a minipig!



Enrichment – Pyramid

We encourage you to provide minipigs with some kind of enrichment. As concluded in the poster on the previous page toys should not replace bedding and rooting material. However, different kinds of toys can be valuable as enrichment. The four groups of minipigs that were observed during this enrichment project showed a particular interest in the bucket in chain and the pyramid. These toys are quite easy to prepare at your facility. See the detailed picture for the dimensions of the pyramid.
You are welcome to contact us if you are interested in further information about enrichment.

Minipig Discussion Group on LinkedIn

The Minipig Research Forum (MRF) is a forum for minipig users all over the world. In order for all these minipig users to connect and to stay in touch a LinkedIn group has been created.

With this LinkedIn Group the Minipig Research Forum has created a forum for scientific discussions and sharing of experiences. Many people have joined the group and several questions have already been discussed.

Join the group on LinkedIn: http://www.linkedin.com/groups?gid=4219925

Meeting calendar

Name	Date	Place
SOT ToxExpo	11-15 March	San Francisco, CA
BTS	25-27 March	Coventry, UK
IAT	27-30 March	Eastern England
Scand-LAS	26-29 April	Trondheim, Norway
AFSTAL	6-8 June	London, UK
Eurotox	17-20 June	Stockholm, Sweden
19th MDO and 12th European ISSX Meeting	17-21 June	Noordwijk aan Zee, The Netherlands