NEWSLETTER 44

AUTUMN 2015

ELLEGAARD
GÖTTINGEN MINIPIGS

10th anniversary of the Minipig Research Forum in 2016
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Clean pigs
for clear results

Dear Reader

Time is flying and, in just a few weeks, we will be celebrating Christmas and soon thereafter New Year, which is a naturally time to look back on the many things that have happened at Ellegaard Göttingen Minipigs.

As "new" CEO, it is my challenge and responsibility to develop and implement a strategy for Ellegaard Göttingen Minipigs, which based on our values – animal welfare, quality, respect and collaboration – can ensure the continuous growth of our company and maintain our good relationship with all our customers. In the future, we will focus on maintaining the Göttingen Minipig as the best characterized minipig in the world, which will include a more focused sales and marketing effort and scientific knowledge sharing plus, very importantly, to focus and initiate many more scientific collaborations to validate the minipig as an important large animal model. For the same reason, I am very pleased to announce the founding of the "Ellegaard Göttingen Minipigs Research Foundation", which will support independent, high quality projects within all fields of research involving Göttingen Minipigs.

Our future focus will also be to ensure that the Göttingen Minipig is the preferred minipig for the development of minipig disease models and transgenic minipig models. We have already taken part in the process of supporting the development and characterization of several transgenic Göttingen Minipig models through our collaboration with PixieGene and recently initiated new global initiatives using the Göttingen Minipig as the founding minipig model. Further, we will focus on branding the Göttingen Minipig and ensure global access to the animals in all significant R&D markets, which will include our already fruitful collaborations with Marshall in the US, OYC in Japan and Woojung in South Korea.

I hope you will enjoy the Newsletter – and wish you all a Merry Christmas and Happy New Year!

Lars Friis Mikkelsen
CEO
Ellegaard Göttingen Minipigs

Follow us on LinkedIn!

Now you can follow Ellegaard Göttingen Minipigs on LinkedIn! Our Company Page on LinkedIn will keep you updated with useful and interesting information regarding our company and the Göttingen Minipig!

New Marketing Coordinator at Ellegaard

In May 2015, Paulina Smirnova Vølund started as Marketing Coordinator for Ellegaard Göttingen Minipigs. Paulina has an MSc in Economics and Business Administration - International Marketing from the University of Southern Denmark, where she completed her master's thesis on "Emptynesters' consumer behaviour and self-realization". After graduation, Paulina worked on marketing for Orkla Foods Danmark before joining Ellegaard. Paulina is devoted to her work and looks forward to providing Ellegaard and customers with the best possible solutions within marketing, support and creativeness. In addition, Paulina serves as the backup function for Order Management when needed, so some of you may have already been in contact with her. Paulina's education and former high level of gymnastics performance have taught her that no goal is achieved without hard effort and focus.

Paulina lives near Ellegaard with her husband and their two-year-old son Viktor. In her spare time she loves to travel, spend time in the garden, paint and play the piano.

We hope that you will have the opportunity to meet Paulina and discuss your Minipig work. You are welcome to contact her if you have any questions regarding marketing: psv@minipigs.dk, direct phone: +45 2941 2349.

We look forward to introducing you to Paulina!

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Update from North America

With demand for Göttingen Minipigs in North America remaining strong, we have broken ground on the construction of our third minipig barrier. The completion of our housing space last year allowed us to significantly increase production and we no longer require extended lead times for minipig deliveries. The new facility will be a second breeding facility and will allow us to maintain two distinct breeding colonies. This redundancy will help safeguard the North American supply of Göttingen Minipigs and help us keep up with the continuous demand.

Marshall BioResources is also excited to announce the launch of our new website, www.marshallbio.com. Our website will provide background data, instructional documents, and general information about all of our animal models including the Göttingen Minipig. We encourage you to register on the site to gain access to all of our materials. We can also provide many additional products and services related to the Göttingen Minipig. This includes biological products and tissues, as well as pre-shipment screening and acclimations. Additional details about our products and services can be found on the website as well, or you can contact us at infous@marshallbio.com.

Construction of our new minipig barrier is underway.

Visit our new website at www.marshallbio.com

Management of Juvenile Minipig Studies

Edward Marsden, BSc, ERT

Associate Director, Scientific Operations, DART and Juvenile Toxicology

WIL Research Europe - Lyon

1. Introduction

Obviously the rat is the preferred and most widely used species for juvenile toxicity studies. However, non-rodent species are required punctually, for example for a pediatric only indication or when the rat is unsuitable due to pharmacological, toxicological or toxicokinetic reasons. A scientific justification for the non-rodent species is typically provided and, in general, one species is required. The dog has been the most widely used species due to its predominance in adult safety studies, but the minipig should be, and increasingly is, considered for both adult and juvenile toxicology programs.

2. Animal procurement – Göttingen Minipig

Pregnant females must be ordered to give birth in the lab since lactating females with a litter cannot be transported. Even newly weaned piglets cannot be transported until 4 weeks of age, so with delivery and a short acclimatization period, it would be difficult to start dosing until 5 weeks of age. Ellegaard requires 3 to 6 weeks notification for the animal order depending on the number of females required. The earlier the order is confirmed, however, the better for logistical purposes for both Ellegaard and the Test Facility and also from a financial perspective because the animals may be obtained at lower cost.

Following an initial experience with primiparous females farrowing on site, we would now recommend that multigravida females are requested for juvenile toxicology studies even though they are more expensive. We found that the farrowing date was more predictable, the piglets tended to be more "vigorous" and litter size tended to be superior. It is also reassuring to know that the multiparous females have already demonstrated good nursing behaviour and milk production at Ellegaard.

After delivery, pregnant females are acclimatized for approximately 3 weeks before the scheduled farrowing date. Batch arrivals are more than likely necessary for pivotal studies (up to 8 litters/batch) in order to optimize study management. This also allows adjustments to be made in the last delivery batch if necessary.

3. Study types

Depending on what pre-clinical safety studies have already been performed in the adult minipig, a pilot tolerability study may be required in juvenile animals of the target age to investigate a maximum tolerated dose. Typically, one or two litters are required to provide 1 piglet/sex/group that will be dosed over a short period (e.g., 2 weeks) so that initial information on tolerability, dose-response (including TK/exposure evaluation), method of administration (parenteral routes), specific observations etc. can be obtained.

A dose range-finding study (with or without a previous juvenile pilot study) would then be recommended. Three or four litters to provide 2 piglets/sex/group to explore dose-response (including TK/exposure evaluation), repeated dosing (e.g., through to weaning), growth, physical development, clinical pathology and terminal endpoints.

Once doses have been selected, the pivotal juvenile toxicity study can be performed. The study design will be customized based on multiple factors such as the age of the intended pediatric population, the pharmacological and any identified toxicological target organ(s), practical constraints with respect to the route of administration etc. Group size is not specified in the regulatory guidelines but typically it is double that used in adult non-rodent repeat dose toxicity studies: N = 6/sex + 3/sex for any reversibility phase. We estimate 3 litters to provide the animals necessary for one group including a reversibility phase. So with one control and 3 treated groups, a minimum of 12 litters should be anticipated.

4. Littering and piglet selection criteria

Everyone working with pregnant minipigs knows that the duration of gestation is 3 months, 3 weeks and 3 days giving a total of 114 days. However, then there is reality! The expected farrowing date is provided by Ellegaard in the delivery certificate (see below) but in our experience with non-induced delivery, the reality is +/- 4 days of the specified date. For this reason, we have asked Ellegaard to also include the number of actual mating dates (number of consecutive days the female accepted the boar) so it is possible to better anticipate the day that conception may have occurred.


ELLEGAARD

ANIMAL CERTIFICATE

Peri-natal monitoring is obviously essential for the future success of the study. The health status of the mothers, including nursing behavior and milk production, and piglets, including mortality/morbidity, litter size, sex ratio and physical/functional development, is closely followed (Fig. 1).

Figure 1: Healthy nursing sow and suckling piglets

In studies where dosing starts shortly after birth, the piglets are pre-selected based on weight and physical/functional development parameters. The piglets receive an intramuscular injection of iron 24 to 48 hours after birth.
Typically, a piglet that is considered acceptable has open eyes rapidly after birth and is capable of standing, suckling and walking. Litter "runts" typically weigh less than 300 g and do not meeting one or more of the above criteria (open eyes or walking) and are therefore excluded from the study (Fig. 2 and 3). In case of surplus piglets and where cross fostering is not possible, the smallest are eliminated whilst taking into account the sex ratio for the study group.

Figure 2: Healthy newborn piglets: eyes open, standing, walking and suckling

The data from 25 pregnant females that farrowed in our facility, give a mean litter size of 8.4 piglets, mean live litter size of 8.0 with a mean of 7.1 piglets per litter meeting the selection criteria. Therefore the majority of piglets meeting the selection criteria are allocated to the study which is essential from an ethical perspective. Piglets not selected for the study were euthanized and frozen prior to examination in the fetal morphology department for potential congenital abnormalities (Fig. 4).

Figure 3: Selected piglets

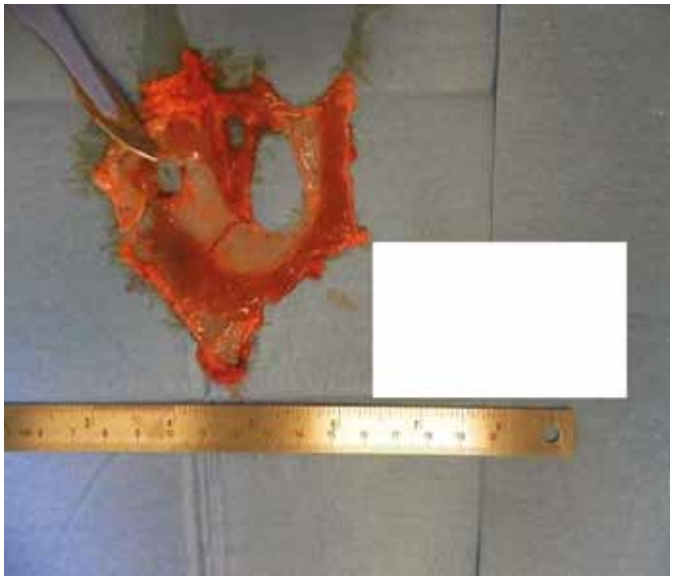

Figure 4: Diaphragmatic hernia for a non-selected piglet detected in fetal morphology lab

Cross fostering is obviously possible if farrowing is synchronized to a suitable degree and the method has previously been described in Ellegaard Newsletter no. 43.

5. Dosing techniques

Oral (gavage), subcutaneous and intramuscular routes pose no particular problems from as early as PND 1 (Fig. 5-6). The minipig should also be conducive to ocular administration since the eyes are open rapidly after birth. The dermal route holds particular challenges if administration starts prior to weaning due to maternal interactions with the offspring. The precautions and procedures required will depend on the age of the piglets at the start of dosing, subsequent body weight gain, type of dermal application performed including the duration of exposure, rinsing, occlusion of the site of administration etc.

The biggest challenge, however, remains the intravenous route and, in particular, infusion. Juvenile piglets can be implanted with a catheter within the first week after birth but the rapid growth of the animals must be taken into account if treatment is to be performed for any length of time (Fig. 7). Once again, maternal interactions with the piglets have to be monitored closely for interference with the infusion jacket etc.

Figure 5: Holding position for oral gavage

Figure 6: Oral gavage PND 1

Figure 7: Piglet with implanted catheter and protective jacket

6. Measurements and observations

Growth parameters including body weight, tibia length and shoulder height can easily be measured as of PND 1 (Fig. 8). Other classical in-life endpoints such as ophthalmology, clinical pathology and cardiovascular examinations (ECG) can be undertaken within the first few weeks of birth (Fig. 9).

Other investigations such as a functional observation battery for assessing neurotoxicity, and immune function assays to assess immunotoxicity are also possible in the minipig.
Blood sampling for toxicokinetics is possible from shortly after birth. Depending on the required blood volume, composite or sequential sampling is possible. For example, 6 microsamples of 80 µL were taken from the same piglet on PND 1 in a recent investigation (Fig. 10 and 11).
Terminal endpoints such as organ weights, macroscopic examination and histopathology are comparable with those performed for adult non-rodent studies.

Figure 8: Growth parameters measured

Figure 9: Piglet undergoing ECG

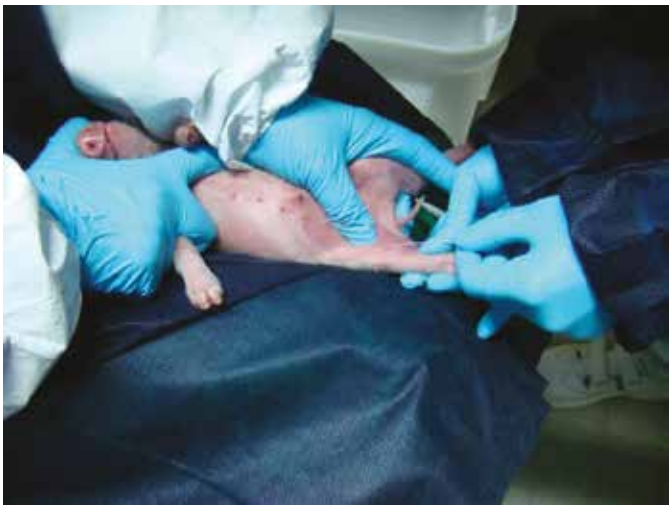

Figure 10: Holding position for saphenous vein microsampling

Fig 11: Collection of blood bead into Microvette tube

7. Conclusions

It is easy to select vigorous healthy piglets for juvenile toxicology studies and perform technical procedures, including dose administration and plasma exposure monitoring, as early as PND 1 in the Göttingen Minipig. The developmental periods are sufficiently short to consider long-term studies (including reversibility) even through to sexual maturation if necessary. The physical size during the minipig postnatal developmental "windows" is very conducive for technical procedures and assessments.

There are some limitations, however, such as availability of historical control data, particularly for very young piglets. This deficiency will be transient though as more studies are performed and experience is enhanced. Intravenous dosing and blood sampling will, however, remain a technical challenge in the minipig, although surprisingly, venous access is easier in juveniles compared with adults. Finally, the advanced growth rate and maturity at birth may lead to other animal models being more comparable with the human for certain biological systems (e.g., neuromuscular and respiratory).

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Poster presentation SOT 2015 San Diego:

Bailey, G., et al. (2015). Microsampling-coupled Bioanalysis in the Neonatal and Juvenile Göttingen Minipig and Beagle Dog

Catheterization of Minipigs

Jacqueline Scotto, Facilities Manager, United States Army Edgewood Chemical Biological Center

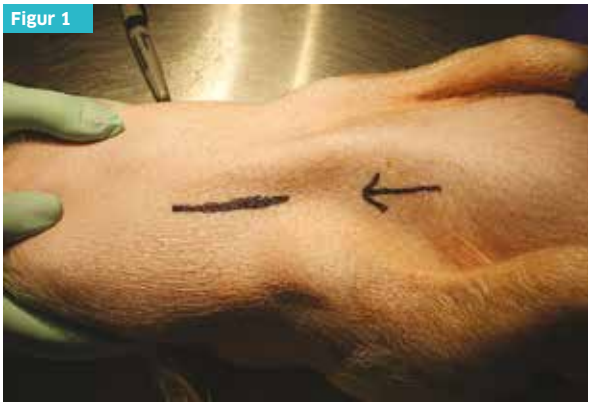
I have been working with Göttingen Minipigs since May 2002, and have performed surgical implantation of indwelling vascular catheters on 1,013 animals. I now have a very high success rate with patency, and with the popularity of using Minipigs in research, I wanted to share the success that we have had. I have taught several different organizations of this surgery and hopefully this will be useful to you.

I use 9.6 F single lumen and 12 F double lumen catheters from Bard Peripheral Vascular, Inc. The double lumen catheters are perfect for when you have to administer a test substance or medication on one side, and draw blood from the other. I use animals that are 7 to 14 kilograms and the 9.6 F and the 12 F are used for each weight range.

Start with the animal in dorsal recumbency. Locate where the jugular groove is in the neck. Make the incision in that divot and you will be able to locate the exterior jugular in minutes (Figure 1).

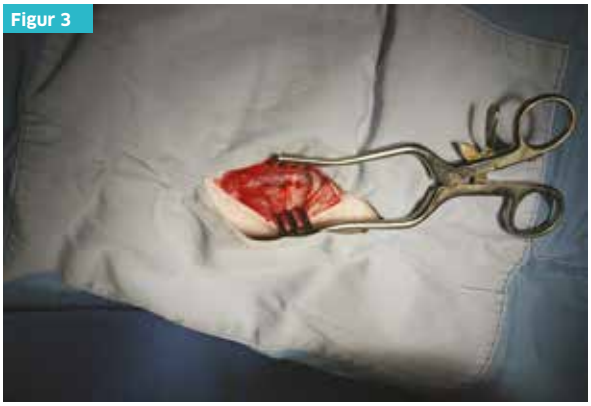
Make an incision that gives you enough room to access the vessel, but not too small so you cannot work efficiently. I make a 5 cm incision and if needed you can always extend this (Figure 2). Blunt dissect and as soon as the muscle layer is opened the exterior jugular should be visible (Figure 3). Clean the fascia from the vessel carefully making sure the vessel doesn't get ripped or cut. Once the vessel is cleared off, place 2 ties cranially. Also, make sure to leave long tails as this will aid in threading the catheter (Figure 4). I use 3-0 silk and I have never encountered a problem, and I use 2 ties due to the size of the vessel. Once the vessel is tied off, rotate the animal just

Figur 1

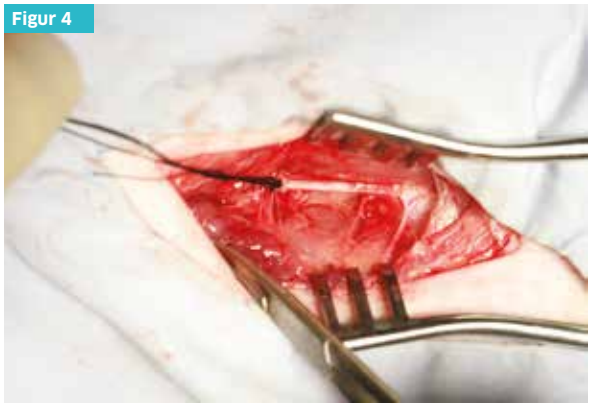


Figur 2

Figur 3

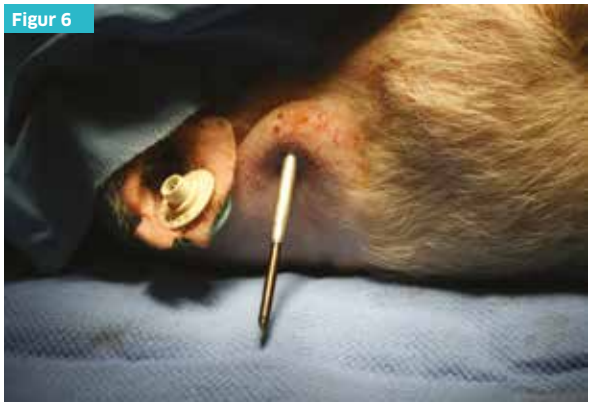


Figur 4



Figur 5

Figur 6



Figur 7

Figur 8

Figur 9

enough for your assistant to have access to prep for the catheter placement.

Tunnel from the incision to the neck, trying to aim for a location that they cannot reach, making a small incision to fit the trocar through the skin (Figure 5 & 6). Thread the catheter through the trocar and pull through leaving only the catheter in place, making sure the cuff enters the skin. Measure the catheter to the Xiphoid process and cut at a 45 degree angle, with sharp scissors to get a clean cut, approximately 6.8 cm cranial from the Xiphoid process (Figure 7).

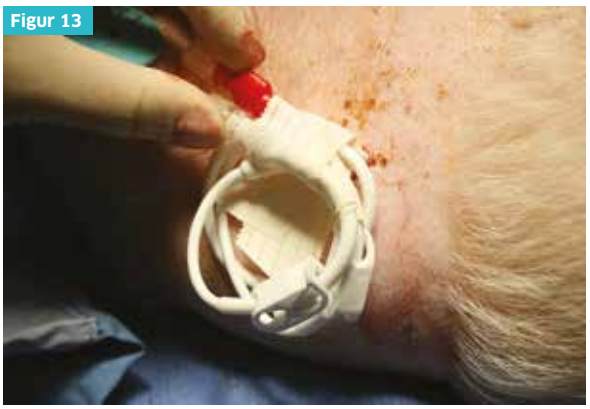
Pick up the vessel using non-traumatic forceps and make a small cut, using micro scissors, at the most cranial point (Figure 8). Insert a vein introducer in the cut that was just made (Figure 9) and advance the catheter all the way in (Figure 10). Place a minimum of 2 ties at the site of catheter entry into the vessel (Figure 11). Check patency with initial placement and after each tie. Use 10 mls of heparinized saline (12,000 units of heparin added to a liter of normal saline) to lock catheter. Close the muscle layer and skin layer. Place a suture at exit site of catheter on back of neck to close skin tightly around the catheter (Figure 12). Coil the catheter and tape in place, so that if the catheter does slip out of the bandage, the pig will not be able to reach it and chew it (Figure 13). Place sterile 4 X 4's with a bead of triple antibiotic ointment on the incisions, wrap with 2 layers of roll gauze, and a roll of 3M vet wrap® being careful not to put too tight. I have an assistant place a hand on the gauze while applying the vet wrap layer (Figure 14).

I usually do surgery five days before they are to be tested and have found to have better patency if the catheter is left alone. Once the catheter is locked at time of surgery, we do not touch them until the day of testing. When testing is done 3-4 weeks after surgery, I will flush the catheters (with 3 mls of heparinized saline) once a week and change the bandage if it is dirty or too tight. After the initial dose of 0.03 mg/kg of Buprenorphine, analgesics are given PRN if animal is displaying signs of pain and antibiotic are not given unless indications of infection.

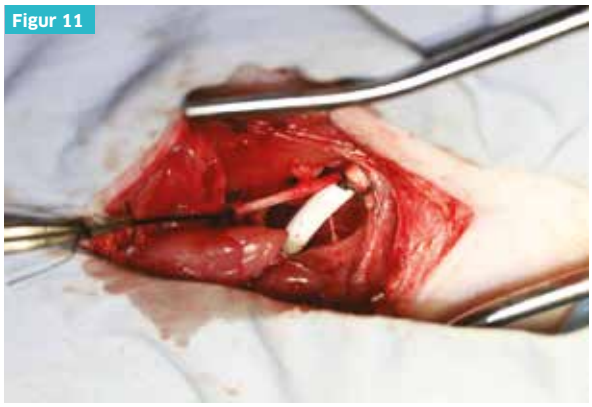
I had to work out many issues before I got to this point, and after working out the process, I have performed this surgery in the same manner and always achieve patency, with no systemic infections, and the pigs recover very quickly.

Figur 10

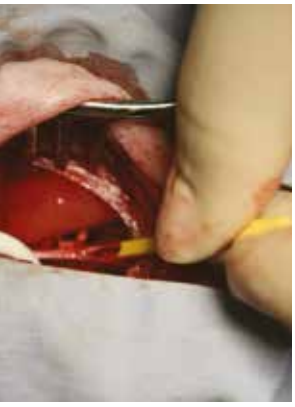
Figur 13



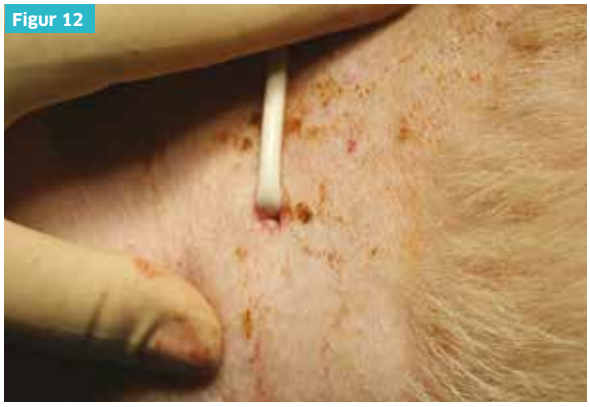
Figur 11



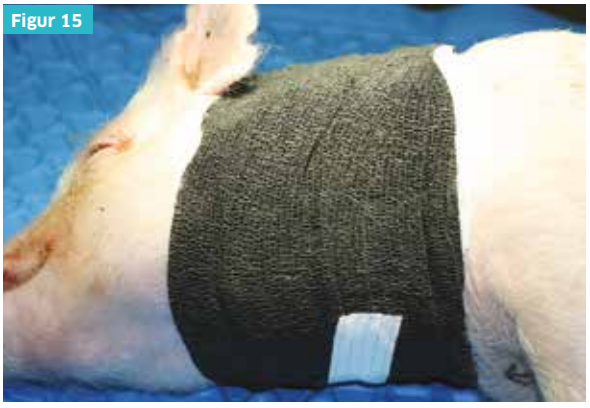
Figur 14



Figur 12



Figur 15



Enrichment of older Göttingen Minipigs

By Ann-Sofie Cæcilie Søndergaard, DVM, Head of Veterinary Services at Ellegaard Göttingen Minipigs A/S

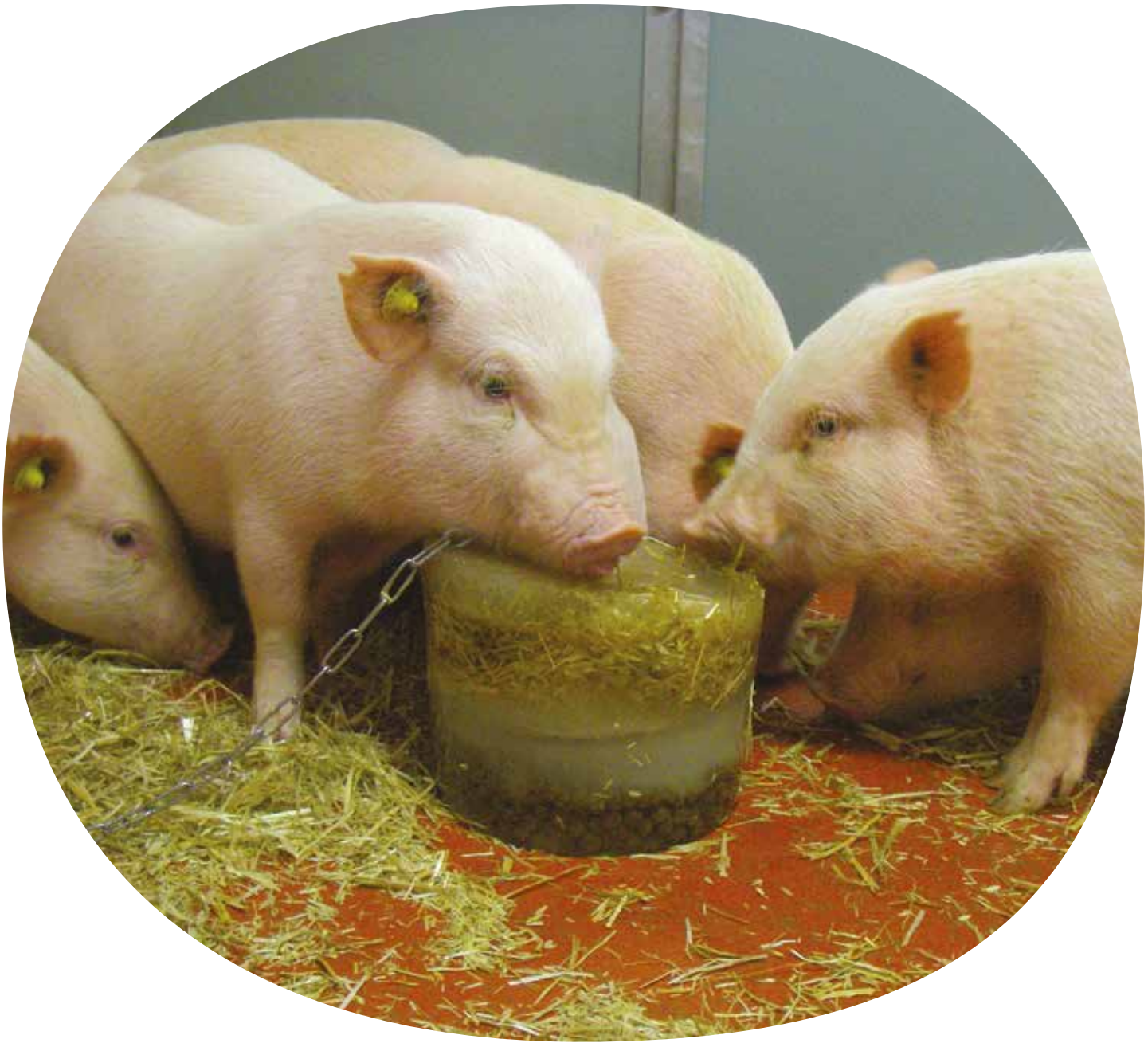
At Ellegaard, we implement a variety of actions and provide different toys as enrichment for our minipigs. This is done to avoid boredom and most of all to give minipigs an opportunity to express different elements of their behavioural repertoire, such as foraging and exploratory behaviour.

We are continuously considering new ways to enrich our minipigs. In every pen, we have basic enrichment such as biting nipples and chains hanging from the sides. In addition, we give them daily walks in the corridor and provide a variety of toys, which are replaced on a regular basis, such as pyramids, towels, balls and biting toys.

From previous in-house projects, we have learned that what works as enrichment for some pigs might not work for others. As an example, observations revealed that older minipigs tend to show less interest in toys than younger pigs. From this, we learned that enrichment needs to be adapted to the age of the minipig in order for it to reach its full potential. Therefore, we choose different actions in different sections of the facility.

We recently increased our focus on the section housing our older pigs, as none of our existing toys really seemed to interest them for longer periods of time.

We know that some of our customers have found it very satisfactory to use ice cubes containing water, yoghurt and a few pieces of chow or apples in them. At Ellegaard however, one has to keep in mind that we have a strict barrier-breeding facility meaning that not everything can enter.



Ice cubes seemed like a good idea, as all of the equipment for the production of such was already inside the barrier (water, buckets). Some of the main characteristics of the objects, which focus pigs' attention for longer periods are that they are ingestible, destructible, deformable and chewable. The "ice block" was designed according to several of these characteristics, e.g. irradiated straw sticking out can be chewed on, the ice itself is destructible/deformable and the food inside is ingestible.

We know that a toy soiled by manure drastically reduces the pigs' interest in the toy. To prevent the ice block from being pushed down into the manure section of the pen, we put the end of a metal chain inside the bucket with water before freezing. After a day or two of freezing, we then had a large ice block with a chain in the end, which could easily be attached to the sides of the pen. The chain needs to be long enough for the ice cube to rest safely on the ground so that the pigs can push it around with their snouts.

How long the ice block lasts naturally depends on the number of pigs interested in it. In pens housing 10–12 older minipigs, the ice block lasted for 1.5–2 hours under constant interest from several pigs. When pigs play with the ice block, the chain attached to the pen side will rattle. This sound and movement of the chain also attracts the pigs and gives any pigs with a lower hierarchal status a chance to play as well.

However, toys cannot replace the value of straw as bedding and rooting material, which should always be available to pigs. Although it is sometimes referred to as enrichment, at Ellegaard we consider it an essential part of a minipig's life, which is why it is available at all times at our facility.

Ophthalmic drug product development:

Why not consider the minipig in your non-clinical strategy for first-in-human studies?

Benoît Ruot, Ph.D., Cert.Vet.Ophth.

Senior Toxicologist, Associate Director, Non-Clinical Safety Program Management

WIL Research Europe

The eye is the second most complex organ after the brain, composed of many different types of cells, made of several cell layers and multiple connections. This complexity is at the origin of the challenges associated with ocular drug development. Ocular drug product development depends upon various elements such as the drug candidate itself (small synthesized entity, biopharmaceuticals, biosimilar, oligonucleotides, vascular endothelial growth factor, etc.), dosage forms and strengths, indications and usage, anticipated frequency and duration of treatment in clinical trials, age range and patient's gender, etc …

With regard to non-clinical safety assessment, regulatory expectations for ocular drugs are not well defined. There are currently no specific guidances (none from ICH, US FDA, PMDA or EMA agencies) to define standards for non-clinical toxicity and pharmacokinetic to support the safety of ocular products for human trials. As a consequence, the applicant may consider applicable general guidelines, such as ICH M3, ICH S6 (for biopharmaceutical) or ICH S9 (for eye cancer) to develop a successful safety plan which, to be accepted globally, take into account expected regional specifics (i.e. mainly taking into account differences between regulatory expectations from Japan, US and EU).

To properly support an initial Phase I clinical study, the plan should be prepared on a careful science-based approach. The first priority will be the demonstration of the absence of systemic toxicology potential of the drug candidate, which can be addressed in a rodent species (the rat for instance), via a route of administration which provides systemic exposure (i.e. intravenous injection, etc.) for a defined duration (at least 14 consecutive days depending on clinical plan). One of the most important (and problematic because of the complexities associated with the eye anatomy and physiology) preclinical testing programs for ophthalmic drugs is certainly ocular pharmacokinetics. Albino (New Zealand White) rabbits are generally the species of choice for preclinical ocular pharmacokinetics testing, and when there is a concern that an ocular compound may bind to melanin, comparative pharmacokinetics studies may be done in species with pigmented eyes (i.e. : Dutch Belted rabbits).

ICH guidelines require the use of two species in non-clinical studies for pharmaceuticals, but with sufficient rationale one species may generally be sufficient for drug candidates intended to be administered by the ocular route. From a non-clinical toxicity perspective, the species selected should be relevant to humans and to the targeted disease. In general and as for other drugs, the selection is a scientific driven process and must be made on a case-by-case basis where the benefits (to humans) are assessed based on scientific evidence such as the target (and the expected pharmacodynamic effects) and also if possible on anticipated (local) toxic effects. Rabbits, dogs, and/or monkeys, are among the most commonly used species to date in the local safety evaluation of ocular drugs, all with their pros and cons (Short, 2008[I]) (such as the anterior chamber depth, vitreous chamber profundity, retinal vasculature, the distance between the ocular surface and internal ocular tissues, etc. …).

The minipig is today a less commonly used non-rodent species in the safety assessment of new ocular drug candidates (Bode et al., 2010)[II]. However this species, fully accepted by regulatory authorities, presents very interesting features which make it a suitable model for studies where direct dosing to the ocular structures is required. Clearly the selection of appropriate method administration procedures to mimic human conditions is a major requirement. Therefore the use of intravitreal (IVT), subconjunctival (SC), or intracameral (IC) injections (to the anterior or posterior segments) is becoming a more and more popular approach to support the development of new candidate, the minipig eye being large enough to perform accurate ocular injections.

Examination techniques

The eyes are examined at regular and defined intervals before and after treatment in unanaesthetised animals (a huge advantage compared to NHP). Adequate restraint is a key factor during the examination of the eye and its per-ocular structure. This examination should be made in a logical sequence, according to internal procedures. Before dilation of the pupils with tropicamide, the orbit can be evaluated for bilateral symmetry, eye-orbit relationship, deformities, presence of discharge, anisocoria… .

After this preliminary inspection, the minipigs are examined using an indirect ophthalmoscope, with interposition of a 20D lens, and a portable slit-lamp. The systems are moved toward the animal's eye so as to obtain an adequate focalization on the examined structures. Large amount of accumulated data on ophthalmological findings in the Göttingen minipig is now available.

Although the minipig can be easily trained/encouraged (i.e. positive training/reinforcement and the use of a clicker) to facilitate eye examinations, animal handling during ophthalmoscopy

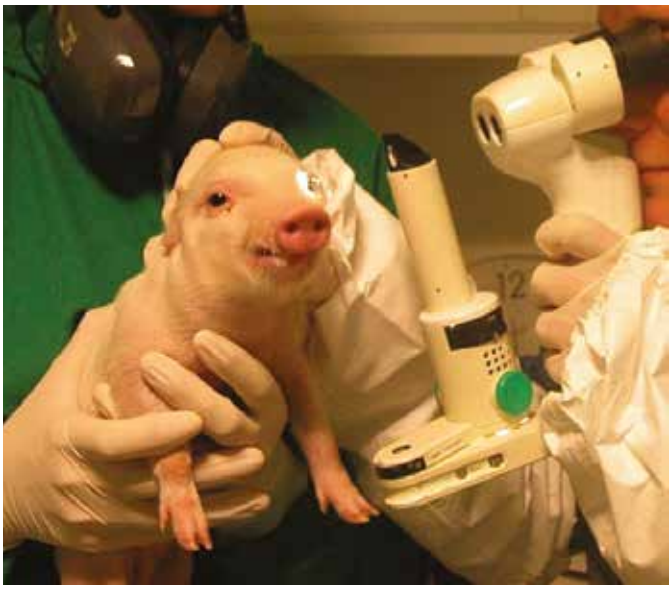


Figure 1: Slit lamp examination in a juvenile minipig

Figure 2: Indirect ophthalmoscopy examination in a young minipig. Depending on their size, minipigs can be held on the arm while larger animals can be put in the sling (after appropriate training).

examination may potentially be an issue; the minipig has very strong extra ocular muscles and upper and lower eyelids that could make ophthalmic examinations challenging (Fig. 1 and 2).

Numerous other examination techniques exist, and most of them require specific instruments and diagnostic procedures. They can require specialized expensive instrumentation and fastidious protocols to achieve reproducible results, such as optical coherence tomography, electroretinography (ERG) or corneal topography imaging all of which can also be applied to the minipig.

Elements of anatomy and physiology which make the minipig an appropriate model for ocular toxicology.

Recently, Sanchez et al. (2011)[iii] have compiled pig eye anatomical descriptions, and compared those parameters to the human eye. The structure and anatomy of the porcine eye appears to have a typical "primate-like architecture" and resembles the human eye in both size and retinal blood supply. This eye has no real macula, but does have an equivalent, identified as the "visual streak" (Fig. 3 - 4). The visual streak is a horizontal region measuring approximately 2.5x15 mm situated slightly above the optic disc, parallel to the horizontal axis, therefore mimicking the macula region in primates, human and non-human (Sanderson and Hubbard, 2002)[iv].

The centre of the visual streak is devoid of major retinal vessels, in analogy with the foveal avascular zone in the human macula, with photoreceptors that mimic the primate macula. The minipig is not an albino animal, but the retina is pigmented and melanin is present in proportions similar to humans. Apart from the architecture of the retinal arteries the vasculature of the porcine retina closely resembles that of the human eye. The pig retina is large, and has a high cone density, as evidenced by the average ratio of rods/cones in the porcine retina at 8:1, compared to that of humans approx. 20:1. Since the 60's, the retina of the domestic pig has been recognized to be more similar to the human retina than that of other larger species such as the dog, or goat (Prince et al., 1960)[vii].

Perhaps most importantly, the porcine model has a holangiotic retinal vasculature (fully vascularized, unlike the rabbit), choroidal blood flow and no tapetum. These advantages make them ideal for ophthalmic research. For example, young animals are commonly used as experimental models for retinal ischaemia. Other areas of active biomedical research using the pig eye as a model include glaucoma induction and ocular drug delivery device development.

The central venous ring of the pig eye is formed by various vessels and occupies the center of the optic disc, making visualization of the lamina cribosa more difficult than in humans. The

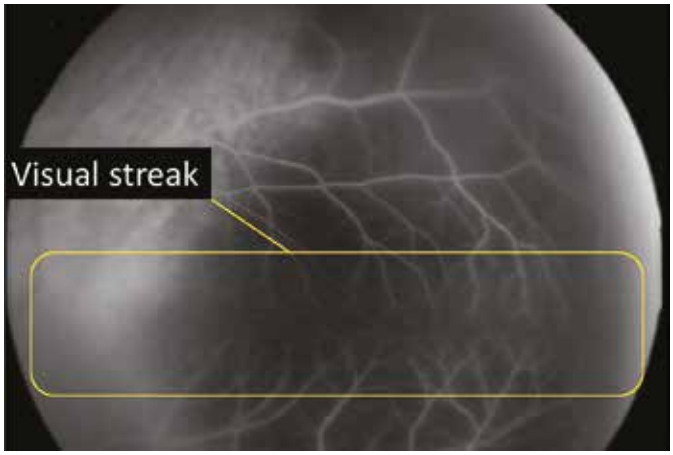


Figure 3: Fluorescein angiography of the pig retina on which the visual streak area is marked. From Håkan Morén (2009)[v]

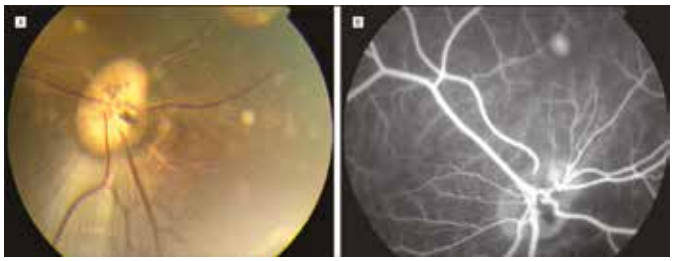


Figure 4: Fundus photograph (A) and fluorescein angiography (B) of a miniature pig retina. Visual streak area is circled in yellow on (B). From Yong Tao et al. (2005)[vi]

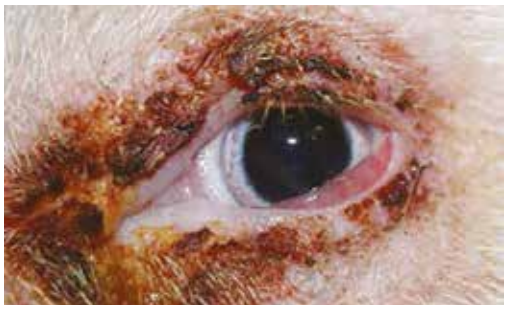


Figure 5: reddish brown mucoid and crusty discharge. From Helmut Ehall (2014) [VIII]

Figure 6: reddish brown mucoid and crusty discharge.

thickness and surface area of the porcine sclera make the porcine eye a suitable model for studying transcleral drug delivery. In addition, the vitreous humor presents with characteristics similar to humans.

However, as for the other species there are some "limitations" in using the Göttingen Minipig. Among others, spontaneous background findings may be present around the eye and could limit local safety evaluation. Hyperkeratosis (probably not associated with Candida infection or chronic dermatitis) can be observed generally around both eyes as "dark brown material" like sun glasses (Fig. 5 and 6). Although hyperkeratosis has absolutely no impact on the general health condition of the animal, the drainage of the tears from the eyes through the nasolacrimal system may be compromised.

Of note, some corneal abrasions associated with conjunctivitis can be observed at eye examinations as the result of the grooming habit of the pig.

Conclusion

In conclusion, the most important factor in the safety evaluation of ocular drugs is the selection of a relevant animal species - approached on case-by-case basis - always bearing in mind the 3Rs! This selection is mainly driven by the type of compound to be developed (NCE *versus* NBE keeping in mind that all information on the formulation development aspects will play an essential role in the definition of the nature and the timing of the pre-clinical studies to be performed prior to the Phase I clinical trial), the target (anterior versus posterior segment, binding of the drug to ocular melanin and the route of administration (topical instillation, IVT, IC…). If the drug candidate - particularly a novel ocular drug - has not been previously studied, ocular route studies in two species (typically nonrodents) would be preferred by the regulatory authorities. However, one species may be sufficient for ocular toxicity studies with proper justification, especially in cases of lack of biologic homology in other species. The Göttingen Minipig should always be included as an option on equal terms with other non-rodent species in the selection of the most appropriate species.

The mini-pig is gaining increasing popularity for various purposes in pre-clinical programs by pharmaceutical companies and contract research organizations, although still suffers from a paucity of immune-phenotyping antibodies to explore the cells of the immune system by flow cytometry, a situation which will improve with time.

NOTES

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Research:

Advanced echocardiography in Göttingen minipigs

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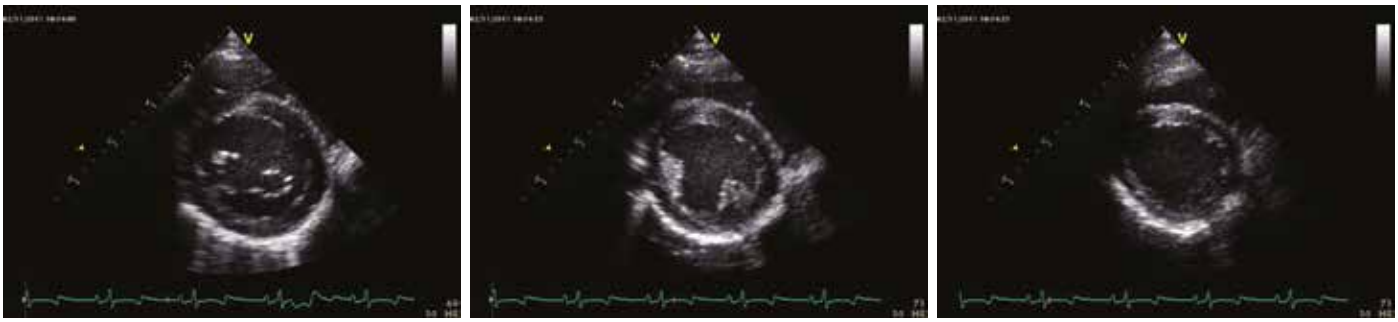

Echocardiographic short axis views of the left ventricle in a Göttingen minipigs. From the left, at the level of the mitral valve (basal view), papillary muscles and apex (apical view), respectively, using two dimensional (2D) transthoracic echocardiography in accordance with human guidelines. Electrocardiography (ECG) is recorded continuously during the echocardiographic examination.

Porcine animal models for cardiovascular disease may be useful for future testing of new diagnostic modalities and promising cardiovascular pharmacological agents that can have major impact for future treatment of human patients with cardiovascular disease. Clinical modalities highly relevant to clinical settings can be used in the models due to the large size of the animal and the comparability between porcine and human anatomy and physiology.

Standardized two-dimensional transthoracic echocardiographic images in accordance with human transthoracic guidelines can be obtained in porcine models. Some echocardiographic views can be difficult to obtain in porcine models, especially the echocardiographic apical four chamber view used for estimation of left ventricular volume and ejection fraction. Therefore validation of alternative echocardiographic methods estimating cardiac function including new advanced technics such as speckle tracking and 3-dimentional (3D) echocardiography is needed. Furthermore influence of anesthesia on echocardiographic evaluation of cardiovascular function in Göttingen minipigs is partly unknown.

A research group at University of Copenhagen has focus on cardiovascular imaging in animal models. A training course including porcine hands on echocardiography will be announced. For further information please contact professor Lisbeth Høier Olsen: lisbeth.hoier@sund.ku.dk

Key references:

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UNIVERSITY OF COPENHAGEN
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The Ellegaard Göttingen Minipigs Research Foundation

We hereby launch the Ellegaard Göttingen Minipigs Research Foundation with the main objective of maintaining and expanding the activities of Ellegaard Göttingen Minipigs A/S by providing funding for scientific research of the highest quality.
In 2016, the Ellegaard Göttingen Minipigs Research Foundation grants in total up to € 100,000 annually to support scientific research that aims to characterize the Göttingen Minipig or promote the development of Göttingen Minipig disease models. In addition, projects that intend to improve animal welfare and/or optimize handling or research techniques as well as educational and communication activities related to the use of Minipigs in scientific research may receive funding.

The Ellegaard Göttingen Minipigs Research Foundation's general criteria for granting research funds are that the scientific content of the application, the qualifications of the applicant and the academic environment of the host institution are at a high international level. The project should generate significant background data and/or ensure knowledge dissemination and promote the use of the Göttingen Minipig in scientific research.
The Foundation has set up a scientific committee to ensure uniform assessment of all project applications. Ellegaard Göttingen Minipigs management team will based on recommendations from the scientific committee allocate grants in accordance with statutory requirements twice a year in February and August.
The project application can be found in our homepage: www.minipigs.dk under "Knowledge Base".

Meeting Calendar 2016

Name	Date	Location
IAT Congress-Institute of Animal Technology	8 -11 March	North of England
SOT ToxExpo - Society of Toxicology	13 -17 March	New Orleans, USA
Juvenile Toxicity Symposium	7 - 8 April	Beerse, Belgium
The Minipig Research Forum	26 - 27 May	Copenhagen, Denmark
FELASA	13 -16 June	Bruxelles, Belgium
JSOT – Japanese Toxicology Society Meeting	29 June – 1 July	Nagoya, Japan
EuroTox - European Societies of Toxicology	4 -7 September	Istanbul, Turkey
SPS - Safety Pharmacology Society Meeting	18 – 21 September	Vancouver, Canada
AFSTAL	12 - 14 October	Nantes, France
AALAS National Meeting	30 October – 3 November	Charlotte, USA
ACT - American College of Toxicology Meeting	6 - 9 November	Baltimore, USA

The Minipig Research Forum Meeting 2015

In 2015, the Minipig Research Forum welcomed more than 80 attendees to its annual meeting in Rome.
Fourteen speakers presented valuable information on the following topics: the use of minipigs in development of anti-cancer products; models of metabolic characterization of test compounds in the minipig; and efficacy testing of medical devices on minipigs.
Among others, there was a presentation on gene expression in different tissues from Göttingen Minipigs during organ development from young to old minipigs.
The programme also included workshops where attendees could share their knowledge and experience on minipigs in the development of anti-cancer products, metabolism and blood sampling, and training, respectively.
Many of the scientific presentations are available by logging on to the Minipig Research Forum website:
http://minipigresearchforum.org/
The organizers also arranged a guided tour through the centre of Rome, where we passed some of the city's most famous attractions. The conference dinner took place in a restaurant with a view of the Colosseum. The restaurant offered copious amounts of delicious traditional Italian cuisine, and the dinner gave the attendees an opportunity to network.

10th anniversary of the MRF in 2016

The 10th MRF meeting will take place in Copenhagen, Denmark, on 26–27 May 2016.
The topics will be:

- Immunology and vaccine testing on the minipig;
- Minipig ophthalmology;
- Minipigs in juvenile toxicity testing.

There will also be workshops and time for networking with other minipig users. The programme will be announced at a later date.
For further details and/or if you wish to give a presentation on any of the topics or if you have related posters, please contact info@minipigresearchforum.org.
We look forward to seeing you in Copenhagen!

Follow MRF on LinkedIn!

The Minipig Research Forum group on LinkedIn is an informative, useful platform where minipig users interact, ask questions and share experiences. Find the group "Minipig Research Forum" and apply for membership. You can follow MRF on LinkedIn – to stay connected and be able to contact other minipig users!

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