Clean pigs for clear results

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New Management Assistant at Ellegaard Göttingen Minipigs

On 1 March 2018 we welcomed Søs Pihl-Poulsen as our new management assistant at Ellegaard Göttingen Minipigs. Søs holds a business language diploma in English and German, and her toolbox further comprises an education within strategic communication. For the past 15 years, Søs has worked as an executive assistant with an IPR consultancy in Copenhagen, where she has carried out all kinds of management support, business administration as well as communication tasks for the top management and the board of directors, including planning and organizing events in Denmark and abroad for customers and employees. Søs has been the anchor of several business projects, has prepared decision papers and business strategy plans and in addition, she has been responsible for the company’s communication comprising various branding, marketing and HR activities. For a number of years at the beginning of her career, Søs worked as a paralegal and administrative assistant for patent attorneys within the field of life sciences, primarily with patenting of food ingredients and veterinary products.

At Ellegaard Göttingen Minipigs, Søs assists and supports CEO, Lars Friis Mikkelsen as well as the management team and our employees, and she will be in close contact with many of our cooperation partners worldwide in connection with the expanding international activities of Ellegaard Göttingen Minipigs.

Søs lives outside Ringsted, a 25 minutes’ drive from Dalmose, with her husband who is a schoolteacher. They have a daughter of 25 who graduated from the University of Copenhagen in 2017 and has launched a career within security risk management. In her spare time, Søs enjoys cooking meals inspired by the French cuisine for family and friends, and she is passionate about her hobby singing in a big gospel choir.

Søs looks forward to meeting many of our partners during the 12th MRF 2018 in Barcelona. Please feel free to contact her at spp@minipigs.dk if she can be of assistance to you.

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Dear Reader

In the beginning of a new year, it is natural to look back on the previous year and not least to look forward to the coming year; most importantly of all, many of our customers and minipig users are now benefitting from the expanding services being provided in our Research Facility. We are now able to provide Göttingen Minipigs with several types of surgically implanted catheters, vascular access ports and not least telemetry devices in addition to providing animal model preparation services, which include both diet and surgically induced animal models.

I am really looking forward attending the Minipig Research Forum 2018 meeting in Barcelona with an exciting scientific program, excellent speakers and lots of opportunities for networking during coffee breaks, workshops and social events. I am sure, that many of you will enjoy meeting new and old friends, colleagues and other minipig users during the 3-day event, which this year is combined with a CONFIRM pre-meeting event – another exiting initiative focusing on the translational value of using minipigs for immunological studies.

Please also be aware of the call for project proposals for grants from the Ellegaard Göttingen Minipigs Research Foundation; as we truly hope to receive lots of exciting project proposals, that all will be evaluated by a Scientific Committee before grant announcement during the MRF meeting.

In 2018, we further hope to initiate the export of Göttingen Minipigs to China and India; several new customers in these two territories are awaiting the finalization of the bilateral negotiations on the export certificates, which hopefully soon will ensure access to Göttingen Minipigs in all major global R&D markets.

Finally, being committed to ensuring that the Göttingen Minipig remains the globally leading minipig – also as background for transgenic minipigs, I am looking forward to introduce you to our transgenic strategy initiative, which will be presented and discussed during coming customer meetings, scientific events and conference attendances; all building on our tradition of working in close partnership with our customers.

Happy reading ...

Lars Friis Mikkelsen
CEO
Ellegaard Göttingen Minipigs

Follow us on LinkedIn!
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!
Head-only perfusion in Göttingen Minipigs using a semi-closed system

By Stéphanie De Vleeschauwer, Laboratory Animal Center KU Leuven, Leuven, Belgium

Introduction

Some tissues like brain and teeth are better fixed for histological analysis when they have been perfused with fixative before submerging them into the fixative. Perfusion of tissues is a well-established and described technique in rodents, but few reports can be found on perfusion in large animals like (mini) pigs. In rodents, the thorax is opened under full anesthesia and a needle is put in the left ventricle to get the fluids in; the right atrium is opened to drain the fluids. Usually the animal is first perfused with saline to flush the blood out followed by a fixative solution to fix the tissues. As these solutions are often toxic, the procedure is done under a chemical safety hood.

Applying this technique to minipigs would imply some major difficulties. Firstly, the volumes of perfusate needed to perfuse a whole pig are very large. Secondly, opening the atrium and letting the perfusate drain into the thoracic cavity would imply lots of spillage and difficulties containing the large volume of perfusate. Thirdly, this technique would require major surgery with thoracotomy/sternotomy to access the cardiac structures. Finally, as minipigs usually do not fit under a chemical safety hood, this technique would implicate safety issues for the operator.

To properly (and safely) fix teeth in Göttingen Minipigs (females, age 33-35 months) we have adopted a technique which we developed previously in domestic pigs to fix the brain[1]. This technique only perfuses the head tissues as it uses the carotid artery as inflow and the jugular vein as outflow. This minimizes the fluid volumes needed. As perfusion is done through cannulas, fluids can easily be contained, and the release of toxic fumes can be reduced to a minimum. A roller pump is used to perfuse at the correct pressure. A schematic overview can be found in Figure 1.

Perfusion technique

Animals are anesthetized with a combination of xylazine (1 mg/kg, Xyl-M® 2%, VMD) and zolazepam/tiletamine (2.5-3 mg/kg, Zoletil 100”, Virbac) IM. A catheter is placed in the auricular vein for venous access. After endotracheal intubation, general anesthesia is maintained with isoflurane (1-1.5%) and the animals are ventilated with a tidal volume of 8-10 ml/kg and a frequency of 10-12 to keep normocapnea. Animals are placed in dorsal recumbency and an incision is made in the right jugular region of the neck. The external jugular vein is isolated for the outflow and the common carotid artery for the inflow cannula. Then, a ligature is placed around both vessels but not yet closed and 15,000 of heparin are given IV through the auricular access. Then, the carotid artery is clamped proximally and a custom-made cannula (made from a standard infusion line) prefilled with saline is inserted and the ligature tightly secured around the cannula. The same is done for the jugular vein, which must be clamped distally during insertion of the cannula. As this cannula is for the outflow it does not need to be prefilled. See Figure 2 for a detail of the instrumented jugular and carotid.

Once everything is secured, the animals are euthanized with an overdose of barbiturates and the distal clamp from the vein is released. Perfusion is done with 5l of 0.9% NaCl containing 10,000 of heparin followed by 5l of 4% PFA through a roller pump with a speed gradually increasing to 20 RPM. The blood,
later saline and PFA are collected directly in containers through the venous cannula. After perfusion, both inflow and outflow tubes are clamped, and the cadaver left untouched for 1 hour to allow further fixation. Then the head is removed from the body and maxilla and mandibula are dissected and the bone is cut in between individual teeth to fix each tooth (+ bone) individually. Samples are preserved at least 1 week in PFA before further processing. Teeth collected in this way are well fixed for histological analysis (Figure 3).

References
1 Musigazi et al. Brain perfusion fixation in male pigs using a safer closed system. Accepted in Laboratory Animals, 2017.

Minipigs as a model for ocular studies

By Fernando Negro Silva, Christian Li, Simon Authier
Citoxlab North America, Laval, Quebec, Canada

Introduction
The interest of the pharmaceutical industry for ophthalmological treatments has been increasing, led mainly by an aging world population. However, the registration requirements, guidelines and safety assessment of those compounds are still challenging. Therefore, refined animal models for toxicological testing are crucial for the development of novel compounds.

Classically, rabbits and non-human primates (NHP) have been extensively used because of the size of the eye and physiological similarities with humans, respectively. Although rabbits have bigger eyes, which allow better evaluation of adverse reactions, their anatomy and cellular structures are relatively different from humans. On the other hand, NHP usage raises ethical questions from the public opinion (1,2). Minipigs are on the rise as a relevant model for ocular toxicological tests. Minipigs are considered the second species with anatomical and physiological functions closer to human (3). Table 1 summarizes the structures that are common between humans and minipigs.

As a model, minipigs have several advantages when compared to NHP, because they are easier to acquire, demand less biosecurity, are easier to handle and cheaper to maintain. Moreover, the public opinion pressure over minipigs used for research is less strict when compared with NHP. The scientific community have explored pig as an animal model for ophthalmic drug development, medical devices, surgical procedures and pathogenesis of ocular diseases. For example, a retina hypoxia model was induced in the pig towards embolization with microspheres. Electroretinogram (ERG) was performed and the authors have showed a reduction in the amplitudes of the scotopic and photopic b-wave, photopic a-wave and oscillatory potentials after embolization (4). Moreover, minipigs have been validated as suitable model for glaucoma research (5).

Vitreous and aqueous humor temperature and pH have been measured in rabbits, monkeys and minipigs in different locations. Some significant differences were observed between location and species. Interestingly, ocular pH appeared to be

<table>
<thead>
<tr>
<th>Structure</th>
<th>Human</th>
<th>Minipig</th>
<th>Monkey</th>
<th>Dog</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harderian Gland</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Nictitating membrane</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Bowman membrane*</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Descemet membrane*</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Lens (thickness)</td>
<td>4 mm</td>
<td>7.4 mm</td>
<td>2.98 mm</td>
<td>7.85 mm</td>
<td>7.9 mm</td>
</tr>
<tr>
<td>Retina</td>
<td>Holangiatic</td>
<td>Holangiatic</td>
<td>Holangiatic</td>
<td>Holangiatic</td>
<td>Merangiatic</td>
</tr>
<tr>
<td>Macula lutea</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Fovea centralis</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Tapetum lucidum</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

*cornea structures
lower in minipigs when compared with monkeys and rabbits. Göttingen minipigs are the main strain commercially available and most commonly used. An increase in the usage of this species was observed in 2010, most likely supported by RETHINK project. Although minipigs have been proved as a preclinical model and are included in the OECD alternative species guideline 409, there are some hesitations in industry to use this species as non-rodent testing. Likely, lack of robust historical data and a steep animal growth curve (that requires more drug substance for longer studies) are some of the reasons. Nevertheless, minipig usage has been growing, mainly in North America.

Results and Discussion

Ocular parameters and electrolytes characterization

Table 2 summarizes the ocular measurements and tonometry of the eyes.

Figure 1 shows representative pictures of fundus from different species. Males (5) and females (2) were averaged together. Mean body weight of the animals was 16.8 ± 0.8 kg.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye globe (g)</td>
<td>4.54 ± 0.23</td>
</tr>
<tr>
<td>Aqueous humour (g)</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>Lens (g)</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>Vitreous humour (g)</td>
<td>2.97 ± 0.24</td>
</tr>
<tr>
<td>Tonometry (mmHg)</td>
<td>10.6 ± 2.2</td>
</tr>
</tbody>
</table>

In order to characterize components in the vitreous and aqueous humor, electrolytes and/or thiol were measured. Table 3 shows the electrolyte parameters and thiol concentration of the aqueous and/or vitreous humor.

Electroretinogram

We have performed electroretinogram in anesthetized pigs treated with compounds that are known to disturb retina homeostasis. Indeed, we observed an enhancement in scotopic a-wave amplitudes in eyes given 5 mg gentamicin (-0.59 to 0.90 log ccds/m2) and eyes give 5 mg glycine (0.41 to 0.90 log ccds/m2) when compared to control eyes. Similarly, scotopic b-wave amplitudes were increased for glycine treated eyes (-2.09 to 0.90 log ccds/m2), whereas decreased amplitudes were observed in eyes given gentamicin (-1.59 to 0.90 log ccds/m2). Eyes given 0.25 mg ICG were mildly decreased at lower light intensities (-0.90 log ccds/m2); whereas decreased amplitudes were observed in eyes given 5 mg gentamicin (-0.59 to 0.90 log ccds/m2), the amplitudes were generally within the control range. Together, these results show that electroretinogram in pigs is an effective evaluation method to access retina toxicity effects.

Further, photopic evaluation revealed a decrease in the photopic b-wave amplitudes in the glycine and gentamicin treated eyes. There were no treatment-related effects on scotopic or photopic a- or b-wave implicit time and latency. Marked reductions in oscillatory potentials (~ 4-fold) and flicker response (~ 3-fold) were noted in glycine and gentamicin treated eyes as well. Log K, which is representative of retinal sensitivity, was reduced in gentamicin treated eyes. In contrast, eyes treated with glycine had a slight increase in the log K. As previously published, all expected effects were observed following intravitreal glycine or gentamicin administration.

Discussion

This study provided a baseline/reference of electrolytes in minipig eyes. Sodium, potassium, chloride and calcium concentrations were comparable with rabbit values in vitreous and aqueous humor. Moreover, similar values in the vitreous were observed in published porcine parameters, with the exception of calcium, where calcium concentrations were 4-fold higher than the values observed in the present work. No phosphorus concentrations or thiol concentrations were found in the literature.

Eyes are considered under high oxidative stress, since their structures are constantly exposed to sun light, oxygen tension (mainly retina) and high metabolic activity. Antioxidant content has been evaluated in retina and other structures. In fact, high content of glutaredoxin 2 (GpX2) was observed in retina, lens and choroid. However, no GpX2 was observed in the vitreous humor, which was expected since this enzyme is mitochondrial and vitreous humor is basically non cellular. Thus, our result of free thiol demonstrates that antioxidant molecule is present in the vitreous humor and it might contribute to protect eye’s structures against free radical damage.

Electrolyte measurement from aqueous and vitreous humors could be a useful parameter in the assessment of ocular toxicity in toxicology studies. Moreover, electrolytes may interact with the compound tested and change its expected properties and behavior.

Furthermore, electroretinogram has been used in the preclinical field to identify toxic effects of ocular new therapeutics or off target effects of systemic administration. We have evaluated the electroretinogram traces in minipigs after intravitreal administration of known compounds that alter retina homeostasis.
In fact, we have observed the expected effects (attenuation of b-wave or oscillatory potentials) when glycine and gentamicin were administered. On the other hand, the effects of indocyanine green were mild. Probably, the low number of animals was not enough to capture an effect.

Glycine is a physiologic inhibitory neurotransmitter in the retina. Indeed, transient visual disturbances were observed in some individuals experimentally administered intravenously with glycine\(^\text{(14)}\), which were followed by a prolonged visual evoked potentials when analyzed by ERG. Moreover, patients submitted to surgery, where glycine was used as an irrigation fluid, showed absence of oscillatory potential\(^\text{(15)}\).

Gentamicin is an antibiotic from the aminoglycoside group, known to have toxic effects in the retina. Indeed, reduction in b-wave has been shown in rabbits after intravitreal or in vitro administration of Gentamicin\(^\text{(16)}\), which was dose-dependent and reversible. This selective reduction may be explained by accumulation of glutamate in the retina, because gentamicin impairs glutamate transports.

The ERG protocol evaluated in minipigs here could be considered in the assessment of retinal toxicity in preclinical toxicology studies.
Figure 4: Glycine and Gentamicin reduces oscillatory potential (A) and gentamicin decreases retina sensitivity (B).

References:
The utility of Göttingen Minipigs for inhalation studies: Data comparison of the respiratory parameters pre-, during and post- the acclimatization phase

By Simon Moore, Director of Inhalation Science and Engineering Safety Assessment, Envigo, United Kingdom

At Envigo, we routinely perform studies on Göttingen Minipigs and in this article we share our experiences of using minipigs for data comparison of the respiratory parameters during the acclimatization phase of minipig inhalation studies.

Introduction
The minipig is increasingly being used as a non-rodent species of choice for toxicological and pharmacological studies due to scientific recognition regarding structural and physiological similarities to humans e.g. for skin, immune and cardiovascular system and increased ethical scrutiny of the selection of non-rodent species particularly the use of non-human primates. Additionally, the regulatory agencies are proactively encouraging pharmaceutical companies to consider the minipig as an alternative species.

Minipigs represent almost 1 in 5 of the 250+ non-rodent animal studies conducted in the last 5 years at Envigo. These are predominantly oral, dermal and infusion routes of administration and are a combination of toxicity, dose range finding, pharmacokinetic/pharmacodynamic and safety pharmacology studies. However, the proportion for inhalation delivery studies is significantly lower at around 1%.

Nevertheless, the introduction of any new non-clinical species to the pharmaceutical industry results in a very cautious implementation approach and this is no exception for inhalation studies. There are a number of contributing factors acting as an obstacle for the introduction of minipigs in inhalation studies including:
- Lack of regulatory non-clinical study acceptance by regulatory agencies
- Lack of minipig inhalation capability amongst the industry
- Lack of inhalation specific background pathology data particularly regarding the respiratory tract
- Previous work for a given disease target or compound class being conducted in other non-rodent species

The selection of any non-clinical species should be based on scientific relevance and any species including the minipig should be actively scientifically deselected prior to progressing to higher tier species. This is particularly appropriate for non-rodent species particularly as dogs and primates are afforded a higher level of protection under The Animal (Scientific Procedures) Act - A(SPA), which is not extended in pigs or minipigs.

Over the last few years Envigo has gradually increased its global inhalation minipig capability and experience after validating the model in 2008 including founding an alternative solution to the lack of inhalation specific background pathology data in the form of collecting respiratory tract from non-inhaled studies.

There are two principle methods of inhalation dosing, whole body and mask exposure. Although Envigo have successfully conducted whole body exposure up to 6hrs/day for several weeks in duration, this article will focus on mask exposure and summarize our experiences of acclimatizing the animals, prior to the exposure phase of inhalation delivery studies.

Acclimatizing the Göttingen Minipigs
Prior to the exposure phase of every inhalation delivery study, the animals are acclimatized to the restraint mask procedure. The objective of this acclimatization or sham dosing procedure is to allow the animals to become gradually acclimatized to achieve superior compliance and animal welfare standards, which in turn enhances the overall study conduct and improves the reliance on the scientific integrity of the study. This procedure minimizes the perceived stress effects associated with mask exposure and increases the possibility of identifying any affects from the administered test material as any subtle changes could be masked by artificially elevated respiratory minute volume (RMV) values if the acclimatization process is not undertaken correctly. The RMV is critical as this value is directly proportional to the delivered dose. This information is pivotal in ensuring that the appropriate clinical doses are calculated and applied.

The acclimatization period can be up to a period of 20 days depending on the duration of study exposure, which can be as long as 240 minutes. A typical example of the acclimatization procedure is presented in Table 1.

Table 1: Example acclimatization procedure

<table>
<thead>
<tr>
<th>Day</th>
<th>Total time of restraint (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Harness only</td>
</tr>
<tr>
<td>2-4</td>
<td>15</td>
</tr>
<tr>
<td>5-7</td>
<td>30</td>
</tr>
<tr>
<td>8-10</td>
<td>60</td>
</tr>
<tr>
<td>11-13</td>
<td>120</td>
</tr>
<tr>
<td>14-16</td>
<td>180</td>
</tr>
<tr>
<td>17-19</td>
<td>240</td>
</tr>
</tbody>
</table>

An expedited acclimatization training programme over 14 days up to 240 minutes has also evaluated with no difference in behaviour between the two regimes being observed.

Picture 1 and 2 show examples of the acclimatization procedure. It was observed that the animals prefer to stand for the first few sessions rather than sitting or lying down. This is contrary to dogs, which usually lie down after a couple of minutes of the start of the first session.
Dosing

The precise dose delivered to the animal using a syringe for oral or parenteral routes can be measured exactly, based on the bodyweight of the animal and the concentration of the solution being administered. With inhalation administration, it is not possible to calculate the ‘dose’ given to the animal in the same way. The animal is presented with an atmosphere concentration of the test article and spontaneously breathes from that aerosol, effectively self-dosing based on the animals own tidal volume and frequency of breathing.

As a consequence, the delivered dose needs to be derived based on an estimate of the air volume inhaled during the exposure period as well as the bodyweight and test atmosphere aerosol concentration. Finally, the proportion of inhaled test article that will enter the lungs is dependent on the particle size. The delivered dose is estimated as:

$$DD = \frac{C \times RMV \times D \times IF}{W}$$

Although the RMV can be measured during certain exposures, the RMV values are usually based on widely accepted algorithms. The most common RMV algorithm[1] contains data from other non-clinical species; the equation does not contain any data from minipigs.

Methodology

For measurement of respiratory function, the EMKA IOX version 2.5.6.4 system uses respiratory inductive plethysmography (RIP) bands, one strapped around the abdomen (above the naval and below the rib cage) and one around the chest and are held in place attached to an under shirt which is worn under an outer jacket. The volume measurements of the EMKA system is calibrated using a fixed volume or calibration where a known tidal volume derived from pneumotachograph flow, with the minipig settled on the dosing table, is simultaneously recorded with the RIP waveforms. The RIP system is calibrated over a period of approximately 10 mins and data captured using the EMKA IOX system at the start of the telemetric recordings.

Analysis of all the captured respiratory data from 6 animals (3 males and 3 females (Bodyweights 25-30 kg)) was made using EMKA Auto 2.8.1.8 and processed with Microsoft Excel, as required.

The following parameters are quantified:
- Tidal volume (TV)
- Respiratory rate (RR)
- Respiratory minute volume (RMV = TV x RR)

The respiratory waveforms were collected, stored and analyzed with the parameters recorded in data bins of 1 to 10 mins on Days 8, 11, 14 and 16 of the 20-day acclimatization phase. Times are taken relative to the start of the acclimatization occasion (T=0). Times prior to this are those occasions prior to the acclimatization session while the animal is still present in the home pen (-1 to -0.67hr) or being walked to the inhalation dose suite (-0.083hr) to commence the acclimatization procedure. Times >0 incorporates several phases - during the acclimatization phase (Day 8 was 1hr and Days 14 and 16 were 3hrs), being walked backed to their home pen and resultant home pen and overnight activity.

Results and discussions

The data presented in Figure 1 show relatively stable RR for the animals whilst in their home pen (mean = 16.1 breathes/min), which increased significantly when being walked to the inhalation dosing suite (mean = 40.9 breathes/min). The mean

![Figure 1: Mean respiration rate data for the males, females and combined values for days 8 and 16](image)
value then decreased to approximately background values before increasing again when being transferred back to their home pens.

The average RR during the acclimatization phase on Day 8 was 25.4 breathes/min which decreased to 20.1 breathes/min after Day 16. However, there was no statistical significance difference between the mean Day 8 and Day 16 data due to animal to animal variability. The data from Days 11 and 14 are not presented as they show similar profiles to Day 8 and 16.

The TV data presented in Figure 2 did not show the same change trend as the RR in Figure 1, when the animals were in their home pens (mean = 130 mL/min) and being transferred to the inhalation dosing suite (mean = 124 mL/min). However, the average TV during the acclimatization phase for Day 8 was 193 mL/min, which decreased to 142 mL/min on Day 16.

Comparison with RMV algorithms

Comparing the RMVs against the Alexander equation\(^\text{2}\) and earlier minipig data generated from Envigo\(^\text{4}\) during restraint procedures (Table 2) found that this dataset produced RMV values that were up to 70% lower than predicted based on the bodyweight, suggesting that this algorithm overestimates the RMV for minipigs and RMV values should be ascertained prior to every study.

However, it must be noted that no minipig data was used in the Alexander equation\(^\text{2}\) derivation and minipigs are much less disturbed, responsive or excitable by staff being present or in the proximity than other species (internal Envigo data in rats, dogs and primates). This has been confirmed by using telemetered minipigs measuring blood pressure and heart rate\(^\text{4}\).

The influence of the RR and TV can be observed for the RMV values presented in Figure 3. The mean predose RMV while the animals were in their home pen was 2.04 L/min, which increased significantly to 6.11 L/min when being transferred from their home pen to the inhalation dosing suite. The mean predose RMV during the acclimatization phase for Day 8 and Day 16 were 5.26 L/min and 3.03 L/min respectively. In general, the RR, TV and RMV values for the female animals were higher than the male animals.

This data is equivalent rat acclimatization data\(^\text{3}\), which show a statistical significant change in RR and RMV parameters during the acclimatization procedure with the increased number of sessions (1 to 3).

Table 2: Mean respiratory minute volume during the restraint procedure

<table>
<thead>
<tr>
<th>Case study</th>
<th>Mean bodyweight (kg)</th>
<th>Time of recording</th>
<th>Approx. respiratory minute volume pre-dose (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexander(^\text{2})</td>
<td>22.2</td>
<td>During exposure</td>
<td>8.5</td>
</tr>
<tr>
<td>ß-agonist(^\text{4})</td>
<td>22.2</td>
<td>Pre-dose (but still restrained)</td>
<td>4.0</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>27.5</td>
<td>During exposure</td>
<td>3.6</td>
</tr>
</tbody>
</table>

For Figures 4 to 6 and similarly to Figures 1 to 3, there is significant variation between data snapshots even when only the mean data is presented. However, there are still trends that can be identified.

The RR value in Figure 4 decreased at approximately 7 hrs post dose when the animal holding room phased lighting commenced and the animals became less active. The values rise slightly in the morning when the animal was found to be moving round its pen when the phased daylight lighting procedure was complete (ca ~20 hrs).

The mean values for the RR, TV and RMV from 2 to 21 hrs post dose at Day 16 were 13.3 breathes/min, 132 mL/min and 1.73 L/min respectively.

Although most data points were easily obtained using the 10 minute data bins, the difficulty with measuring RMV data from minipigs is the animals natural tendency to snuffle. This behaviour occurred more often in the home pen and meant that not all data was measureable.
Conclusion
Envigo has demonstrated that RMV measurements can be undertaken in the minipig whilst being acclimatized to inhalation procedures up to four hours. The mean RR, TV and RMV decreased with the number of acclimatized exposures (42%), however, it should be noted there was inherent animal-to-animal variability amongst the 6 animals. The same level of variability between animals has also been observed in rats.

The RMV of the minipig is 70% lower than predicted by the Alexander et al RMV equation, therefore, it is recommended RMV determination should be undertaken prior to every study to ensure that an accurate delivered dose can be calculated until a species specific RMV equation has been calculated. Furthermore, it should be noted when collecting data for minipigs, the data needs to be thoroughly reviewed and parameters changed as some of the data may have to be excluded due to their inherent snuffling procedure.

References
5 Moore S et al (2017). Rat respiratory minute volume comparison of snout-only plethysmography studies collected during the acclimatization phase. 53rd Congress of the European Societies of Toxicology.
Porcine surgical disease models for translational research
(Porcine model of renal autotransplantation)

By Eiji Kobayashi
Department of Organ Fabrication, Keio University School of Medicine, Japan

Introduction
In the previous Ellegaard Göttingen Minipigs Newsletter No. 49, the author has introduced the ‘SDG’ pig model(1), the main points are as follows. The SDG models are experimental animals in which disease is induced through surgery (S), drugs (D), and/or genetic modification (G). In many cases, these models are developed to study the efficacy of various medical supplies candidates. Generally, the development of drug and genetically modified porcine models are expensive and time-consuming processes. On the other hand, surgical porcine models can be produced by using healthy minipigs through surgery alone. To generalize to humans, it is vital to use experimental minipigs as porcine models to induce disease through same surgery method for human. Therefore, these models require the cooperation between surgeons who treat humans and specialists who treat laboratory animals with sufficient training. To create an animal model, the experimental animals should be used by a well-organized expert group who minimize the animal number used for training based on a detailed training program(2).

The author has proposed the 3C’s principles. The 3C’s stand for: Curriculum: in the surgical learning process a well-designed curriculum is essential. Competence: Competency-based learning allows the trainees to learn procedures at their individual pace, targeting predefined competencies. Clinical performance: this term was defined for clinical surgeons. It can also be adapted for biomedical researchers, where performance is manifested in data quality within the frameworks of research projects. In addition, it is essential to fully understand the background of human clinical issues to be solved by this disease model.

Following introduction of “Porcine disease models undergoing surgical operation for translational research”(3), a Göttingen Minipig model with diabetes treated through total pancreatectomy, this paper introduces the renal transplant model.

Minipig model in development of new medical device used for organ transplantation
In recent years, drugs with extremely clear targets for diseases, such as molecular targeted drugs and antibody therapeutic drugs in molecular biology development and drug development is progressing. However, even in times like this, the reasons for the failure of products in human trials are often associated with lack of efficacy in Phase II and III. In preclinical practice, verification of effectiveness as well as verification of toxicity and safety is becoming more important than ever, especially development for medical device, studies using minipig is indispensable due to the size is well-suited for the development of device. From such a background, the US Food and Drug Administration (FDA) has issued a draft guidance “General Considerations for Animal Studies for Medical Devices”(3). On the other hand, I would like to explain the clinical background. In recent years, the development of organ transplantation therapy has become an era that save many patients with organ dysfunctions. However, serious shortage of donor organs is coming closer. As a solution, we have tried to use organs from circulatory cardiac patients. Pig models have attracted a lot of attention for the development of preservation solutions and organ perfusion devices(4 and 5). From such a background, it is required to generate stable minipig organ transplantation model.

Practice of minipig model with autologous transplantation
Generally, in vivo studies of organ transplantation, donors and recipients are needed. However, there is a problem which is inbred is available in small animals but not experimental minipig. Based on the previous described clinical requirement and problem of using experimental minipig, the author ① use the kidney clamped circulatory as donor kidney (left kidney), ② take this kidney to the back table, reflux storage solution, ③ autograft it in the same individual. Finally, ④ remove the opposite side kidney (right kidney). The author has developed such pig model who is providing guidance on it. This model is a model that emphasizes on the animal welfare point of view such as reduction of animal numbers, and no immune response by allogeneic organs.

Experimental animal
Used Göttingen Minipigs and young farm pigs (20-22kg and N=10). After intubation by general anesthesia, insert tubing for infusion into the right jugular vein and blood sampling.

Surgical technique

Table 1. The flow of the whole surgery
[Generation of ischemic kidney graft]: After laparotomy with a midline abdominal incision, clamp the left renal artery vein at one time with a blood vessel clip to make renal ischemia assuming cardiac arrest. Until the ischemic time (usually within 30 to 60 minutes), freeing the donor kidney, the left renal artery and vein to the base. Here, systemically administered 1 cc of heparin, clamped the abdominal aorta with upper and lower left renal artery, the left renal vein was side clamped to the IVC trunk, the renal artery was collected in the Carrel patch type in the graft kidney, secures distance for re-anastomosis renal vein and detaches and remove the graft kidney.

[Back table and preservation]: From the renal artery cannulation side to the back table, verify various rinsing liquid and organ preservation solution from the height of 1 m of water column (Figure 1).

The reflux kidney 'mottled' by the circulatory arrest. In preclinical studies, medicines such as thrombolytic agents mixed in various flushing liquids are tested.

[Recipient]: While preserving the isolated kidney, prepare for put-in of donor kidney by applying 4-point suture with 5-0 nylon to the abdominal aorta part of the isolated site of the same individual's left kidney. When storage time is reached, put the graft kidney in, and make an anastomosis by matching 4 points to the Carrel patch part of the graft renal artery. First, perform continuous suture to the rear wall, and then perform continuous anastomosis to the anterior wall side (Figure 2).

Since the blood vessels that have been removed and cold preserved tend to contract, first put on four points of surgical stature and put on (parachute anastomosis method). Rebuilt Artery carefully by continuous anastomosis with using Carrel patch. At this point, re-apply the blood clip to the peripheral side of the renal artery, and release the blockage of the abdominal aorta. The anastomosis of renal vein is performed by nodular anastomosing both ends with 6-0 nylon, then perform continuous suture to the front wall, invert the anastomotic part and perform continuous suture to the rear wall (Figure 3). After completing the anastomosis, blood flow resumes.

Venous continuous sutures are easy to make a stenosis at the last knot suture. While tugging the left and right support yarns, tighten it till they are lightly drawn together. Fit the ureter with a single node (approximately from 8 to 12 needle stitch) with 6-0 nylon needle carefully. Then remove the right kidney that has been preserved. At the end, close the abdominal once hemostasis at the transplant site is confirmed (Figure 4).

[Post-operational evaluation]: After surgery, collect the blood over time from the indwelling catheter in the right jugular vein and measure the parameters (BUN, Cre) of renal function. After surgery, the renal function changes according to the effect of protocol, flush solution and preservative solution. If treatment for ischemic is strong, postoperative renal function cannot be obtained as primary non-function (PNF), and it will be equivalent.
to both nephrectomized model. Based on past experiences, if PNF is judged on Day 2 and Serum BUN > 100 and CRE > 5.0, of course it is possible to manage by hemodialysis, however from the animal welfare point of view, we recommend that pathology verification of the grafted kidney should be performed after euthanasia.

Discussion
Treatment for end stage renal failure dramatically improved the quality of life of patients with renal failure due to the progress in hemodialysis, peritoneal dialysis, and renal transplantation treatment. Among them, kidney transplantation treatment is said to have not only remarkable advances in patients' QOL and prognosis, but also medical economic advantages. However, the worldwide shortage of brain dead donor is prominent, while on the other hand there is no doubt that it depends on living donors. Under such circumstances, clinical efforts are being made to make cardiac arrested organs to be implantable by mechanically refluxed.

Laboratory pigs are used in many experiments of mechanical reflux of cardiac arrested organs which is a very early evaluation method by ex vivo perfusion. However, in most cases the transplant is not actually been performed. Autologous kidney transplantation models in dogs(6) and pigs(5) have been reported in the past. Autologous transplantation is useful in excluding rejection model as well as using inbred rats model for syngeneic transplantation. However, the models have being used in the past is a model repeated exposure to anesthesia, or a model using continuous anesthesia. The author developed this new model, for generating ischemic renal graft from the clinical ischemic time, and then taking out the graft and investigate the effects of so-called rinse liquid and resuscitation liquid by mechanical reflux.

References:
3 FDA General Information General Considerations for Animal Studies for Medical Devices October 14, 2015.
Evaluation of stress and acclimation to new environments, procedures or equipment in Göttingen Minipigs using behavioral and physiological stress indicators

By Jennifer Baszczak, Linda Panepinto, Temple Grandin, Felipe Berard, Kumari Smith
1 Lovelace Biomedical, Albuquerque, New Mexico, USA
2 P&S Farms, Masonville, Colorado, USA
3 Colorado State University and Grandin Livestock Systems, Fort Collins, Colorado, USA

Introduction
Swine, including domestic farm breeds and miniature breeds, are increasingly utilized in biomedical research because of similar anatomical and physiological traits to humans. Despite the benefits of swine models, specific accommodations have been made to work with laboratory swine due to their particular social, behavioral and husbandry needs. It is well established that swine are highly social and intelligent animals that require substrate for successful behavioral management because of their hard-wired rooting behavior (Ellegaard et al. 2010; Grandin 2014, Panepinto 2014). Therefore, if the swine model is being used as a dermatologic or surgical model, the research/veterinary group must make thoughtful considerations to prevent irritation, bleeding or infection of the wound site. Single-housing on slatted hard flooring without substrate may have been the standard alternative to maintain the integrity of a wound care dressing and bandage. However, barren enclosures, single housing and hard surfaces without substrate do not provide cushion, thermoregulation, rooting or social opportunities (Grandin 1986, 2014, 2015, 2016; Panepinto 1986, 2001, 2009, 2014, 2015, 2016; Smith and Swindle 2006). To that end, it is important that animal welfare and behavioral studies are conducted to assess an animal’s wellbeing within the specifications set forth by a research environment.

This paper describes a collaborative effort of animal vendors, equipment manufacturers, researchers, veterinarians and swine behaviorists in developing a protective jacket for swine which will endure manipulation, be easy to apply and remove by handlers, minimize stress, and provide comfort. We believe that this team approach of consulting experts in the field ensures the highest standard of care for our research animals.

New environments, procedures or equipment can be major stressors for an animal (Ellegaard et al. 2010; Grandin 1986, 1997). Both short term and long-term stress factors have been shown to interfere with an animal’s normal physiological parameters resulting in compromised immune responses and reproduction cycles (Grandin 1986; Swindle et al. 1994). Chronic stressors include long term single housing and housing swine in barren environments with no substrate or manipulanda limiting opportunities for animals to express species appropriate behaviors. Acute stressors include short term restraint for blood draw without prior conditioning or transport (Grandin 1997). Both types of stress factors have the potential to reduce the wellbeing of the animal and therefore should be adequately identified and addressed.

To assess the psychological wellbeing of swine, a simple behavioral scoring system is used (Table 1) for gauging the animal’s emotional state upon arrival to a facility and subsequently, throughout study procedures. To evaluate the animal’s physiological state, salivary cortisol samples can be collected using SalivaBio’s Children’s Swab method.

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
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<tr>
<td>1</td>
<td>swine approaches the observer after 10 minutes</td>
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<tr>
<td>2</td>
<td>swine approaches the observer between 6 and 10 minutes</td>
</tr>
<tr>
<td>3</td>
<td>swine approaches the observer within 5 minutes</td>
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The goal of this project is to evaluate stress in swine during conditioning, placement, and wearing of a specifically designed protective jacket that is durable, thermoregulating, and comfortable for use in medical and research procedures. Through communications with Lovelace Biomedical veterinarians and principle investigators, Marshall BioResources (animal vendor), Lomir BioMedical (equipment manufacturer) and swine behaviorists (Temple Grandin and Linda Panepinto) refinement efforts were two-fold. First, to evaluate two types of Lomir custom designed protective jackets for swine during dermatology or surgical care and secondly, to assess the welfare of the animal during the process of conditioning and wearing the jacket using behavioral and physiological stress indicators.

Lovelace Biomedical collaborated with Lomir BioMedical to design a swine protective jacket to assess ease of use, comfort, and durability. Neoprene fabric was used for the jackets’ material for flexibility and comfort (Mino et al. 2013). Using Lomir’s swine measurement form located on their website (https://www.lomir.com), each pig was measured for establishing a well-fitted protective jacket. Two jackets were developed using the same neoprene material but with different closure types to assess ease of use for the handler when applying or...
removing from the pig as well as durability and long-term wear (Images 1 and 2).

From consultations with well-known animal behaviorists on acclimation techniques to new equipment and procedures, such as jacket placement, several factors were identified:

1. **Start with the best**
   - Swine should be purchased from an animal supplier with a reputable animal welfare program and dynamic behavioral management program that includes
     - frequent handling of weanlings
     - social housing
     - training animals
     - acclimation of animals to novel experiences on their own terms
   - Communicate with animal supplier for selecting previously socially housed swine for ease of transition to a new facility
   - Continue social housing compatible swine in pairs or groups

2. **Gain the animal's trust**
   - Upon arrival to the new facility, immediately begin conditioning swine to humans to gain their trust, using positive reinforcement
     - use gentle tone when interacting with naive swine
     - enter pen slowly
     - sit on pen floor
     - allow swine to inspect care staff
   - Conditioning swine to novel equipment on their own terms such as, allowing each pig with pen mate to inspect and explore a new area or novel piece of equipment (e.g. sling or protective jacket)
   - Clicker or target training swine for
     - increasing their confidence
     - reducing their startle response
     - providing mental stimulation
     - ease of handling

3. **Structural Enrichment**
   - House swine with substrate rather than smooth flooring for
     - traction, as unsecure footing causes stress
     - providing comfort to joints
     - assisting in thermoregulation
     - encouraging hard-wired rooting behavior
     - bedding depth of at least 0.1016 m (about 4 to 5 inches)

4. **Comfortable Fitting Equipment**
   - A swine projective jacket should be designed with the following
     - soft, comfortable quick drying material
     - smooth closures, as rough edges can irritate
     - form fitting

**Methods and Materials**

For this evaluation, four male Göttingen Minipigs approximately 5 months old with weights ranging from 9.3 kg to 14.4 kg were pair housed with original pen mate from Marshall BioResources upon arrival after a veterinarian health assessment. The minipigs were housed in accordance with the Guide (NRC, 2010).
Swine pairs were housed in a 3.048m X 6.096m enclosure (10’ X 20’) with aspen wood shavings approximately 0.1016m (4 to 5 inches) deep as depicted in Image 3.

All animals had access to water ad libitum, fed twice a day and pens were cleaned daily with new substrate added as needed. Both enclosures included a variety of sensory enrichment, with a minimum of 4 hanging and 4 loose manipulanda per pen, rotated weekly for novelty. Behavioral Scores were recorded upon entering the enclosure prior to beginning any interaction or acclimation sessions as described in Table 1.

Acclimation Period

• To humans
  From day 2 post arrival, human acclimation sessions began following the recording of individual behavioral scores. No sessions were conducted on weekends. After the swine approached and moved forward to appear purposeful in making contact with the observer (usually with their snout), the observer could attempt to touch the swine under the jaw or on the sides of their body

• To environment
  Within 5 days after arrival, swine were given “walk-a-bout” sessions with pen mate in the center isle of the animal housing building to explore and increase competency of environment which has been shown to reduce fearful behavior

Training Period

• Clicker training began once each animal was acclimated to the handler
• Each pig was taught to touch a target (a plastic spoon or a PVC pole approximately 0.25m to 0.75m long), follow the target to a floor scale, hold snout to target for up to 10 seconds and return to home pen on cue (Image 4)

Lomir Protective Swine Jacket Acclimation

• After swine were compliant with training >80% of the time when given the cue by the handler, both swine jackets were introduced for acclimation. Jackets were placed on the pen floor during AM feeding for swine to inspect on their own terms and removed by the end of the day

Saliva Cortisol Collection

• Cortisol samples were collected from each animal before feeding using Salimetrics collection swabs in the AM and PM over the course of this study and 3 days after jacket evaluation. The manufacturer’s guidelines for collecting saliva was modified from the SalivaBio’s Children’s Swab Method with the following modification for collection with swine (Image 5)
  1. The familiar human handler entered the enclosure and sat on the pen floor
  2. Swine were randomly selected for individual saliva sample collection
  3. The handler held both sides of the sampling swab provided by Salimetrics Saliva Lab to allow the animal an opportunity to mouth the swab voluntarily until saturated with saliva
  4. Then the swab was inserted into a pre-labeled vial, sealed, and immediately placed on ice. Within an hour of collection, all samples were stored in a -80°C freezer until shipped to Salimetrics for analysis
  5. Swine were rewarded with high value food enrichment after collection

Results

• The 4 male Göttingen Minipigs took less than seven days to acclimate to their new environment and human handlers based on their behavioral scores of 3, for three days consecutively
• All swine were clicker trained and compliant to targeting cues >80% of the time within 14 days post arrival
• Swine showed no signs of stress using behavioral scoring for placement and wearing of either type of protective jacket (Images 6 and 7)
• Handlers noted that jacket #1 with a zipper plus Velcro closure remained on swine for the duration of assessment and jacket #2 with Velcro only was removed by the end of the day by swine
• Cortisol analysis for this project is in progress and results are expected to be published later this year
Conclusion

Communications in the refinement of devices and procedures used in animal medicine and research should include the animal vendor, veterinarians, animal behaviorist and/or manager, principle investigators, equipment designers, and users. Beginning with a reputable animal vendor that is proactive in their commitment for enhancing animal welfare begins the foundation for reducing stress when animals transition to a new environment or novelty. Swine experts recommend gaining the animal’s trust prior to beginning new procedures to decrease behavioral signs of excitability and fear. When assessing the welfare of the animal and progress of their acclimation to novelty, it is important to remember, individual behavioral responses may vary between animal subjects. Using a simple scoring system can assist in identifying fearful or timid animals that can benefit from a modification in their conditioning regime as well as evaluate the animal’s progress over time. In addition, for collecting cortisol to assess acute stress levels in individual swine, saliva collection is a less invasive technique and shows a stress response within minutes after an event. Swine in well-designed jackets behave normally and with a durable jacket closure system will remain on the animal during social housing.

Acknowledgements

Lovelace Biomedical would like to thank the following personnel who helped in the acclimation, training, and excellent care of these animals: Denise Marks, Theresa Garcia, Melisa Kalas, and Nannette Davidson, as well as Steve Allen for his photography of swine and equipment. We would also like to express our sincere gratitude toward Teresa Woodger and Jill O’Connor from Lomir BioMedical Inc. for their design and development of two types of swine jackets for assessment, and to Nicole Navratil and Michelle Salerno from Marshall BioResources for supplying the wonderful animals and for providing endless communications in our collaborative effort to improve the lives of research animals.

References


Lomir BioMedical Inc. https://www/lomir.com

Lovelace Biomedical https://www.lovelacebiomedical.org


Salimetrics Salvia Lab. SalivaBio Children’s or Infant’s Swab Collection Method. 5962 Laplace Court, Suite 275, Carlsbad, CA 92008.


New scientific publications on the Göttingen Minipig

Ellegaard Göttingen Minipigs A/S gives high priority to collaborative projects that aim to better characterize and validate the Göttingen Minipig as a translational animal model and which facilitate and refine the use of the minipig in research projects and safety testing. Please contact us if you have an idea for such a collaborative project. Below is a list of a few recent articles on the Göttingen Minipig.


THE 12TH MINIPIG RESEARCH FORUM

16-18 MAY 2018 IN BARCELONA, SPAIN

The MRF meeting offers exiting presentations on the use of minipigs in research and drug development.

THIS YEAR’S MAIN TOPICS ARE
- Species selection
- Analytical methods & biomarkers
- Imaging & devices
- Disease models

3 WORKSHOPS
- Analytical methods
- Species selection
- Tips & tricks

Poster presentations and time for networking with other minipig users
Already now we accept posters; please send your scientific and/or technical poster as pdf-file to contact@minipigresearchforum.org
The deadline for submission is 15 April 2018

REGISTER NOW FOR THE 2018 MINIPIG RESEARCH FORUM

Registration deadline is 1 May 2018
The registration fee is EUR 350 including get-together-evening, lunch on Thursday and Friday, coffee breaks, social event and gala dinner

GET-TOGETHER-EVENING

Wednesday 16 May 2018

Prior to the actual start of the MRF, we would like to invite you to a get-together-evening.
We will host an evening buffet with food and drinks from 06:00-10:00 pm.

We do hope to see as many of you as possible, so please keep this in mind when booking your flight.
Get-together-evening will be at the same place as the venue.

For further information regarding the MRF 2018 registration, meeting details, preliminary programme, venue and accommodation, please contact us at contact@minipigresearchforum.org or visit www.minipigresearchforum.org
The programme for the meeting consists of a number of scientific sessions on the following topics:

### SPECIES SELECTION

| Species selection – a CRQ perspective with reference to the minipig | Andy Makin, Citoxlab |
| Non-rodent species selection to assure human relevant safety assessment | Susanne Mohr, Roche |
| Use of minipigs to support non-clinical safety of human pharmaceuticals: Update after RETHINK | Jan-Willem van der Laan, Medicines Evaluation Board |
| The selection of non-rodent species for drug toxicity testing in Lundbeck | Qikun Zhuang, Lundbeck |

### ANALYTICAL METHODS & BIOMARKERS

| Porcine biomarkers in the field of inflammation | Peter Heegaard, Danish Technical University |
| Developments in blood collection: working with newborn Minipigs | Mike Burges-Wilson, Envigo |
| Stop Interfering!! Effect of sample quality and study processes on minipig biomarkers | Fiona McClure, GSK |
| Liver and kidney biomarkers – recent findings in Gottingen Minipig models of atherosclerosis and NASH | Henrik Duelund Pedersen, Ellegaard Gottingen Minipigs |

### IMAGING & DEVICES

| Evaluation of embolization devices and drug-eluting embolics in swine | Julien Namur, Archimmed |
| Preclinical evaluation of bioabsorbable scaffolds in minipig vasculature: imaging technologies to evaluate device performance, safety profile and structural integrity of the implant | Louis-Georges Guy, AccelLAB |
| The Gottingen Minipig as an animal model for dental implants | Benjamin Pippenger, Straumann Group |

### DISEASE MODELS

| The use of the minipig as a model in non-clinical wound-healing studies | Trine Starostka, Citoxlab |
| Digital technologies in preclinical CV research – applications in large animal models | Lena Schramm, Bayer |
| The pig as model for human influenza infection – porcine immunology toolbox and perspectives | Kerstin Skovgaard, Danish Technical University |
| The CRISPR-Cas9 Minipig – A transgenic pig to produce specific genome editing in selected tissues | Martin Kristian Thomsen, Aarhus University |
| Development of a urinary tract infection model in Gottingen Minipigs | Jeffrey Fernandez, Janssen |

### WORKSHOPS

- Species Selection
- Analytical Methods & Biomarkers
- Tips & Tricks

### POSTER PRESENTATIONS

For further details about the meeting, please visit the Minipig Research Forum’s website: www.minipigresearchforum.org
Please contact the MRF if you have any questions: contact@minipigresearchforum.org

We look forward to seeing you in Barcelona!
The Minipig Research Forum Steering Committee

**AWARD TO THE BEST POSTER!**

Present your poster and win free registration to next year’s MRF meeting 2019!
We invite any posters with technical or scientific content about minipigs.
Please contact: contact@minipigresearchforum.org

**MRF website!**

Have a look at the MRF website: www.minipigresearchforum.org and register for the meeting in Barcelona!

**Follow MRF on LinkedIn!**

The Minipig Research Forum group on LinkedIn is an informative and useful platform where minipig users interact, ask questions and share experiences. Apply for the MRF LinkedIn group membership by sending us an email: contact@minipigresearchforum.org
You can follow MRF on LinkedIn to stay connected and to be able to contact other minipig users!
Minipig Research Forum
Pre-Congress Meeting

The CONFIRM initiative is delighted to invite you for the Pre-Congress Meeting at the MRF venue, on Wednesday the 16th of May 2018 with the following program:

14:00  General Meeting of CONFIRM and what is the aim of CONFIRM
      Presentation and update of Step-1: Interlaboratory Validation Study
      Plenum discussion of options for Step-2 initiatives

15:00  Coffee Break and networking

15:30  Presentation “The translation potential of porcine disease models from an immunological perspective” by Professor François Meurens, Oniris, Nantes, France
      Presentation and description of the Step-1 Interlaboratory Study Design, by Linda Allais, Charles River, Lyon, France

16:45  Closing remarks

To participate, please sign up when registering for the MRF meeting on www.minipigresearchforum.org member login site. If you already have signed up for the MRF meeting, then send an e-mail to contact@minipigresearchforum.org to sign up for the Pre-Congress Meeting.

To learn more about the CONFIRM initiative, visit https://minipigs.dk/knowledge-base/the-confirm-initiative/
INTERLABORATORY STUDY PROGRAMME:
CALL FOR PARTICIPATION

One major goal of the COllaborative Network For Immunological Safety Research in Minipigs (CONFIRM) is the coordination of interlaboratory studies relevant to immunological safety evaluation in Göttingen Minipigs. A multi-step interlaboratory study programme is being elaborated.

**Step-1** is an interlaboratory validation study to demonstrate that reproducible results in Göttingen Minipigs can be independently generated by several laboratories to achieve better regulatory acceptability. The study plan will be purposely restricted to state-of-the-art objectives: measurement of standard toxicological parameters, biobanking, and immunological safety endpoints (TDAR assay: anti-KLH IgM & IgG ELISA; standard lymphocyte subset analysis; histological examination of main lymphoid organs/tissues). One reference immunosuppressive agent (daily oral cyclosporine) will be used as a benchmark in 4-5-month-old Göttingen Minipigs (3 animals/sex/group) treated for 4 weeks with a 2-week recovery period. Every participating laboratory will be requested to formerly agree to the study plan and methods after an open discussion to reach reasonable agreement. Optional endpoints can be added to the study plan provided the core study plan is strictly followed. Results will be published collectively by representatives of all participating laboratories.

**Step-2** will follow a different approach: evaluating the feasibility and relevance of innovative techniques and read-outs to improve nonclinical immunological safety evaluation and further demonstrate the predictive value of Göttingen Minipigs. The Step-2 framework will include several independent studies, each focused on one goal/area, either to address dedicated safety evaluation strategies related to reference immunotoxicanents (e.g. immune check-point inhibitors, vaccines, recombinant cytokines, small immunomodulating molecules…) or to qualify and validate novel readouts or models (such as immunogenicity assessment, immunogenomics, novel cytometry or cell imaging techniques, development of transgenic animals, predictive assays of pseudo-allergic reactions…). Discussions on proposed outlines of step-2 studies will start during the next Minipig Research Forum to be held in Barcelona in May 2018. This will be a genuinely research-focused approach thriving on close interactions between participating laboratories.

The CONFIRM initiative would be happy to count on your active participation to all or selected parts of this interlaboratory programme. You can express your interest by sending an e-mail to: HDP@minipigs.dk.
The Ellegaard Göttingen Minipigs Research Foundation – call for project proposals

We hereby invite applications for the Ellegaard Göttingen Minipigs Research Foundation with a submission deadline 15 April 2018.

The main objective of the Ellegaard Göttingen Minipigs Research Foundation is to maintain and expand the activities of Ellegaard Göttingen Minipigs A/S by providing funding for scientific research of the highest quality.

The Foundation grants in total up to € 50,000 annually to support scientific research that aims to characterize the Göttingen Minipig or to 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(s) and the academic environment of the host institution(s) 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 May and in December respectively.

The project application form can be found in our homepage: www.minipigs.dk under “Knowledge Base” and should be submitted to Henrik Duelund Pedersen, CSO, Ellegaard Göttingen Minipigs by e-mail to hdp@minipigs.dk no later than 15 April 2018.

Meeting Calendar 2018

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<tr>
<th>Name</th>
<th>Date</th>
<th>Location</th>
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<tr>
<td>ToxExpo and SOT Annual Meeting</td>
<td>11 - 15 March</td>
<td>San Antonio, Texas, USA</td>
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<tr>
<td>IAT Congress</td>
<td>20 - 23 March</td>
<td>Northern England</td>
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<tr>
<td>Minipig Research Forum (MRF)</td>
<td>16 - 18 May</td>
<td>Barcelona, Spain</td>
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<td>TALAS</td>
<td>4 - 7 June</td>
<td>Bangkok, Thailand</td>
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<td>AFSTAL</td>
<td>13 - 15 June</td>
<td>Reims, France</td>
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<td>EUROTOX</td>
<td>2 - 5 September</td>
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<td>GV-SOLAS</td>
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<td>SPS</td>
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<td>LAVA-ESLAV-ECLAM</td>
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<tr>
<td>ACT</td>
<td>4 - 7 November</td>
<td>Palm Beach, Florida, USA</td>
</tr>
<tr>
<td>Bio-Europe</td>
<td>5 - 7 November</td>
<td>Copenhagen, Denmark / Malmö, Sweden</td>
</tr>
<tr>
<td>AFLAS</td>
<td>28 November - 1 December</td>
<td>Bangalore, India</td>
</tr>
</tbody>
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