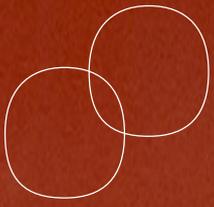


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GÖTTINGEN MINIPIGS

MAGAZINE



ELLEGAARD ••
GÖTTINGEN MINIPIGS

Dear reader

Since the last publication of Göttingen Minipigs Magazine in March this year the world has changed significantly and might never be the same. The COVID-19 crisis that hit the entire world within weeks has created many until then unknown challenges, but also lots of proactive and creative solutions, but my thoughts are first of all directed to the many who have suffered the loss and sickness of relatives and loved ones.

Crisis management became very evident and as emphasized in the article on p. 3-4 a must for all institutions and organizations. I am glad and proud that we managed to maintain our business

almost as usual, managed to maintain the daily care of our Göttingen Minipigs, and even managed to secure safe and secure shipment of animals to customers in both Europe and outside Europe. This was only possible because of dedicated and motivated colleagues and business partners, to whom I am more than thankful.

The new situation also highlighted the need for virtual meetings and extended use of social media. Today, having virtual meetings with global stakeholders seem the norm and participation in webinars and virtual conferences a part of the daily routine. Again, may I thank all the presenters and participants at our very successful webinars - it is amazing that several hundred participants attend again and again. We will continue to plan interesting and relevant webinars even after the COVID-19 crisis.

I hope you will enjoy reading our magazine. Let us all stay together in this unusual situation, although with a certain physical distance.

Stay safe!

Lars Friis Mikkelsen, CEO
Ellegaard Göttingen Minipigs A/S



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COVID-19 put crisis management to the test overnight

From time to time organizations face unpredictable events, which can have a higher or lower invasive impact. To deal with the risk of drastic changes in the way of conducting business and considering the unpredictability of local, global, and even online events, it is vital to have a crisis management plan. Also, crisis management often requires decisions to be made within a short time frame and often after an emerging event.

When a crisis occurs, the ideal situation is not to handle it unprepared and based on surprise, which often results in rash actions and decisions. Over the years Ellegaard Göttingen Minipigs A/S has focused increasingly on developing, maintaining, and implementing a crisis management plan covering the entire organization. "Though you cannot predict everything that might happen, you ought to always consider which incidents are more or less likely to emerge - and most importantly: What will you do if they occur. When you have been through this exercise, actions in case of a crisis will undeniably be more deliberate" says Lars Friis Mikkelsen, CEO at Ellegaard Göttingen Minipigs.

In addition to their crisis management plan, they recently established a Risk Management Task Force with representatives from their key inhouse disciplines such as Production & Facilities, Veterinary Services, Business Development, Finance, and IT. The role of the task force is to uncover and assess potential risks, and prepare contingency plans, should an event occur.

Make your own crisis management plan

1. Assess your risks

Identify potential crises that would disrupt your business function and/or processes. Work with members of leadership, your crisis response team, and other key stakeholders and list all relevant threats and vulnerabilities that could impact the company. These might include negative media coverage, social media shitstorms, product recalls, cyberattacks, data breaches, etc.

2. Determine the business impact

A business impact analysis (BIA) is a way to quantify the potential impact of a business-disrupting crisis, and can reveal a variety of potential effects, including:

- Customer dissatisfaction or attrition

- A damaged reputation in the public eye
- Lost or delayed sales or income
- Increased expenses (e.g. overtime labor or expedite shipping)
- Regulatory fines

A BIA is important to make sure your organization truly considers every angle of a threat, and it helps when arguing the value of crisis management plans towards stakeholders.

3. Identify contingencies

Identify which actions will help your company to respond effectively to each crisis scenario. Which steps are required to resolve a given crisis, what resources are required, and how can employees help. For example, a crisis plan for a product recall may require help from IT and logistics to determine how to fix the problem, while customer service, sales, and public relations work together to answer customer questions and maintain the company's good standing.

However, one event that very few organizations had prepared for, was the global COVID-19 pandemic that spread through continents within a few months. "Contingency plans are great to have, but my best guess is that very few facilities, had a plan for what to do when a pandemic, like we are witnessing with COVID-19, hits you" says Jan Lund Ottesen, Vice President LAS at Novo Nordisk A/S, as he reflects on the year 2020 to date. As many other companies, they did not have a crisis management plan covering global pandemics, so they had to improvise a lot and use elements from different crisis management plans.

Both Novo Nordisk and Ellegaard Göttingen Minipigs had two primary areas of focus: First, securing the health and well-being of all employees. If possible, working as much as possible from home or alternately working in shifts for e.g. animal caretakers, "to mitigate the risk of a COVID-19 quarantine affecting all employees at the same time" Jan Lund Ottesen argues.

Second, communicating as much as possible with information relevant to every employee's working situation. Jan Lund Ottesen explains: "In a situation like this you simply cannot over-

communicate. That said, it is very important that you ensure a consistent and timely communication all the way from the CEO to the employees focusing on: 1) What we know; 2) What we will do; and 3) How will this affect you."

Already having strict biosecurity and quarantine regulations, Ellegaard Göttingen Minipigs even took extra measures to prevent an internal outbreak. Lars Friis Mikkelsen elaborates: "All physical interaction between units was stopped immediately. Even joint lunch breaks. Only employees with crucial functions entered the premises, such as animal caretakers, minimum order management and logistics personnel - and our drivers had no physical interactions with customers during deliveries."

Both Novo Nordisk's and Ellegaard Göttingen Minipigs' crisis management plans were put to the test following the governmental lock-downs attempting to control the spreading of disease. About executing their crisis management plan, Lars Friis Mikkelsen evaluates: "Be proactive, creative and act responsibly. With this in mind, you can handle any crisis. It is amazing what a team of any size can accomplish if you stand together - even apart."



4. Build the plan

Once you have determined an effective contingency for each potential crisis, clarify the plans with relevant stakeholders. Key employees can provide insight into available resources and potential hurdles. Certain crisis scenarios may also need input from outside parties, such as contractors and partners that work closely with your business. Keep in mind any relevant regulatory requirements, and determine how you will continue to meet them, even in the midst of a crisis.

5. Familiarize users

It is important that all employees understand their roles during a crisis. Remember, stress and panic can make it difficult to remember your role in a crisis response.

First, ensure that your stakeholders have the information they need. During the tense moments of a crisis, people require very quick access to straightforward information. Consider ways to

distribute a crisis plan quickly and effectively, incl. access to up-to-date documents, incident reporting, contact lists, etc.

Second, train stakeholders frequently on your crisis management plan. Stage regular tests and rehearsals to ensure that every individual is familiar with the plan, can respond confidently, knows where to get additional information, and understands his or her role.

6. Revisit the plan frequently

Once your plan is written, approved and has been tested, be sure to revisit it frequently. It is vital to keep the plan up to date, especially as employees join or leave the company, new technologies are implemented, and other changes occur. Consider reviewing and testing the plan at least a few times a year to keep the content fresh.

Source: "6 steps to create a crisis management plan" by RockDove Solutions Inc., rockdovesolutions.com

Göttingen Minipigs and other laboratory animals in COVID-19 research

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There is a need for good animal models for research in COVID-19. Not all experimental animals are susceptible to the disease, but the animal species in combination can provide insights into the pathogenesis, prevention and treatment of the disease. Minipigs are among the experimental animals used in this research.

The need for animal experimentation in COVID-19 research

The global impact of the COVID-19 pandemic has included the closing down of whole sections of society in 2020 with major human and economic consequences leading to a rapid increase into the research of the biology of the disease, its treatment and the development of new vaccines. Consequently, at least 90 different vaccines against COVID-19 are under development (Callaway, 2020), besides an unknown number of different types of new treatments. Although it is tempting to save time during such a pandemic by moving directly to testing in humans instead of first performing animal experiments, this is a dangerous approach, especially as the vaccines and treatments will probably be applied to a very large part of the world's population. Therefore, animal experimentation still plays a key role in this research, and will continue to do so for years to come. However, the choice of animal species is crucial, as not all species are directly

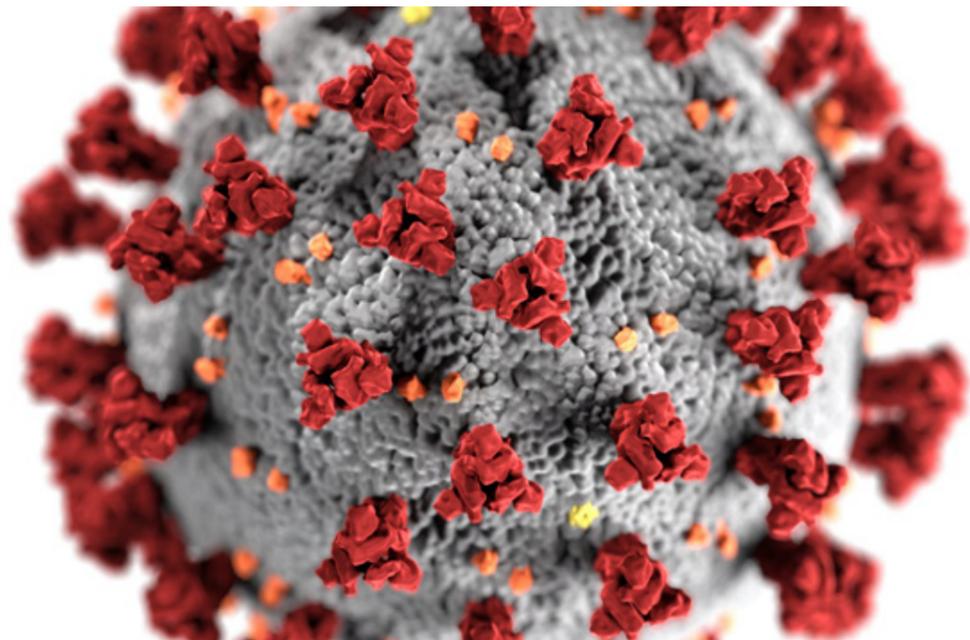
susceptible to infection with COVID-19 (Singh et al., 2020), and the development of new animal models are trial and error processes, slowly leading to greater insights.

Susceptibility among animal species

The severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the coronavirus behind COVID-19, uses the angiotensin-converting enzyme 2 (ACE-2) to penetrate the cells. This blood pressure regulating surface-associated enzyme is found in cells in the lungs, heart, arteries, intestines and kidneys, where it breaks down angiotensin II, thereby reducing blood pressure (Dworakowska & Grossman, 2020). The surface structure of ACE-2 is all-important in determining whether or not an animal species is sensitive to infection with SARS-CoV-2.

Ferrets, cats and hamsters

A study using wild-type animals showed that SARS-CoV-2 can



Picture 1
Illustration of SARS-CoV-2, the coronavirus behind COVID-19.

EARA global overview of how animals are helping in Covid-19 research

Click on the icons to see where research using animals has contributed to a biomedical study. Click on an icon in the key to hide it.



(Source: WHO Global research on coronavirus disease database and European Animal Research Association sources)



Illustration 1

Interactive map by the European Animal Research Association (EARA) showing global COVID-19 related activities within research, drug and vaccine development involving research animals.

Access the interactive map:
<https://bit.ly/COVID-19-research-map>

infect the upper respiratory tracts in ferrets and the nose, throat and respiratory tracts in cats. This, in particular, makes in addition to Syrian golden hamster (Eisenstein, 2020) the ferret a valuable animal model, which is well-recognized by researchers carrying out lung infection studies.

Dogs and pigs

Dogs, on the other hand, are only slightly susceptible to infection, and chickens, ducks and pigs are all resistant (Shi et al., 2020). The lack of susceptibility of dogs and pigs is probably also due to their limited expression of ACE-2 on the surface of airway cells (Zhai et al., 2020).

Non-human primates

It has also been possible for scientists to use rhesus macaques, which can develop COVID-19 (Deng et al., 2020). However, cynomolgus macaques did not reproduce the respiratory disease seen in rhesus macaques, but still cynomolgus macaques could be used to evaluate preventive and measures against SARS-CoV-2 infection, as virus replication has been seen in the respiratory tract (Rockx et al., 2020), but produces much milder disease than is typically seen in humane patients.

Transgenic animal models

Mice are also naturally resistant to COVID-19, but fortunately there is still a remaining small population of ACE-2 transgenic mice that was developed for SARS research after the

epidemic outbreak in 2003, and these transgenic mice are also sensitive to the current coronavirus (Jiang et al., 2020). Similar transgenic models in pigs and other large animals will probably be possible to obtain via the CRISPR technique.

It is worth noting that although many animal species do not develop COVID-19, or only with weaker symptoms than humans, they will often still develop neutralizing antibodies. Thus, a Chinese study has detected antibodies in 14.7 percent of all wild cats in Wuhan (Zhang et al., 2020) and a later Italian study has also detected antibodies in both cats and dogs, and with a higher prevalence in dogs from homes with humane COVID-19 infections (Patterson et al., 2020).

Göttingen Minipigs in COVID-19 research

Although most COVID-19 research is currently conducted using susceptible strains in ferrets, transgenic mice and non-human primates, pigs are also used in COVID-19 research. As the pig immune system shares many similarities to the human immune system, pigs are used for testing antibody response for new vaccines and documented, that vaccine boosters are more effective than single vaccination in experimental pigs (Graham et al., 2020). The pig will further be a desirable large animal model, as most societies want to use as few non-human primates as possible in research.

Danish Göttingen Minipigs coronavirus treatment study

Although pigs and minipigs are not directly susceptible to SARS-CoV-2 virus and they do not develop symptoms of COVID-19 disease similar to humans, a Göttingen Minipigs study has been initiated at the research facility at Ellegaard Göttingen Minipigs to assess the safety of a potential treatment for COVID-19. The study is performed in collaboration with the University of Copenhagen and SoftOx, that has developed an antimicrobial solution intended to treat infections in the skin and with a technology that has a documented effect on virus and bacteria. The goal of the study is to test whether the SoftOx solution can be nebulized for inhalation to prevent and treat respiratory tract infections caused by viruses as well as other microbes. After thorough animal testing in Göttingen Minipigs, including

documented mucosal and lung tissue safety, and necessary regulatory approvals, the aim is to conduct the first clinical trials in patients with current respiratory tract infections.

Final comments and conclusions

Experimental animals are useful tools for research into COVID-19's pathogenesis, treatment and vaccination. Some animal species are directly susceptible to COVID-19, such as ferrets, while others, such as mice are only susceptible when they are transgenically altered. No single animal species fully reflect COVID-19 infections in humans, and therefore a combination of small and large animals is needed. Although minipigs are not susceptible to spontaneously developing COVID-19, they can be usefully used for testing various treatments for the disease. In addition to the examples mentioned here, minipigs have also

been used for vaccine development and secondarily for the development of new ventilators and respirators that can be used on human patients. Here it is an advantage that the size of the pig lungs makes them comparable to humans, so that results and equipment developed in pigs can be immediately applied in humans. This is clearly an advantage in situations where fast results are in demand. A number of other large animals, such as cows and llamas, are used in research into the treatment of COVID-19 with neutralizing immunoglobulins. The age of the experimental animals is probably also a parameter that one should consider as the cytokine storm is worst in elderly patients with COVID-19. Therefore, a whole range of experimental animals are needed to gain insight into the pathogenesis, prevention and treatment of COVID-19.



Picture 2 (page 8 top), picture 3 (page 8 bottom), picture 4 (above)
Safety assessment of the SoftOx solution by ventilated inhalation in an anaesthetized Göttingen Minipig.

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A model for continuous bladder infusion in Göttingen Minipigs

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At Charles River Laboratories Copenhagen, a surgical model was established in Göttingen minipigs that allows continuous infusion into the bladder of minipigs for at least 5 consecutive days by an exterior pump and simultaneous urinary sampling through a 3-way Foley catheter. The feasibility of the model was assessed clinically and by urine analysis.

Initial considerations

The aim was to establish a model to enable instillation/infusion of solutions into the bladder by a catheter and an external pump, thus allowing extended infusion of any chosen pattern (continuous, pulse dosing or bolus) and flow-rate, whilst simultaneously allowing easy sampling of urine. First attempts to fixate a transurethral catheter proved difficult and this approach was not suited for continuous infusion. A transabdominal approach was therefore chosen by which a catheter was tunneled subcutaneously to a secure position on the animal. In contrast to a transurethral catheter, a transabdominal procedure can be applied to both sexes. A 3-way Foley catheter (Rüsch, 18F, 40 cm) was chosen to allow simultaneous test article infusion and urinary sampling.



Picture 1
Emptying of the bladder during surgery. Two (2) stay sutures are placed to hold the bladder in place during the procedure.

Anesthesia

Six female minipigs (24,5-26,5 kg) were anaesthetized using an intramuscular injection (1 mL/10 kg bodyweight) of a mixture of Zoletil 50[®]Vet., Virbac France (125 mg tiletamine and 125 mg zolazepam), 20 mg/xylazine/mL (6.25 mL), 100 mg ketamine/mL (1.25 mL) and 10 mg butorphanol/mL (2.5 mL). After induction of anesthesia and preparation of the animals for surgery, the animals were intubated, and anesthesia maintained using 2% isoflurane in oxygen. Artificial ventilation was not necessary.

Surgical procedure¹

Incision sites were shaved and disinfected thoroughly with soap and water. Ethanol and finally iodine were applied. Local analgesia (lidocaine 10 mg-adrenalin 5 µg/mL) was injected prior



Picture 2
Caudal to the two stay sutures a purse string suture is placed. The catheter will be inserted into the bladder through an incision within the purse string suture.



Picture 3
The roman sandal suture is continued along the catheter for as long as possible.

to incision. Sterile drapes were used to secure aseptic access to the entire surgery field.

The animal was placed in dorsal recumbency throughout the procedure. Caudal to the umbilicus a 10 cm midline incision was made through the skin and linea alba was identified. Using forceps, the linea alba was elevated followed by a stab incision. Using scissors, the incision was extended carefully avoiding damage to underlying organs. The bladder was located and stay sutures were placed at a ventro-lateral position. At the desired location (cranio-ventrally) for entry of the Foley catheter into the bladder, a purse string suture was placed. In the skin of the right flank an incision was made, and the catheter was tunneled subcutaneously and entered the abdominal cavity at a latero-cranial position to the bladder through a separate stab incision. Using a large gauge needle and a syringe, the bladder was emptied, and an incision was made into the bladder within the purse string suture. The Foley catheter was inserted, and the balloon inflated with saline. The catheter was secured within the lumen by tying of the purse string and with a roman sandal suture. The bladder was attached to the abdominal wall using absorbable sutures on the ventral surface. The incision was closed in three layers and the complete surgical procedure required less than an hour.

Post-surgical treatment

For post-surgical analgesia, dermal patches with fentanyl were applied just prior to surgery (50 µg/hour for pigs weighing up to 25 kg and 75 µg/hour for pigs weighing more than 25 kg) for up to 72 hours. In addition, the animals were given an

intramuscular injection of meloxicam 5 mg/mL (0.08 mL/kg) just prior to surgery and once daily for the following two days. The animals were treated with methadone 10 mg/mL (0.02 mL/kg) 3-5 hours after the surgery. Further, amoxicillin 150 mg/mL (1 mL/10 kg) was administered by intramuscular injection the day before surgery, on the day of surgery and once daily for the following three days.

Post-surgical recovery

The animals recovered well from anaesthesia and spontaneous urination was observed soon after surgery and throughout the study. Three (3) animals recovered less rapidly and decreased activity, subdued behaviour and reduced appetite were noted for 9 days in a single animal and for 3 days in two animals following surgery. Additional pain medication was applied as appropriate following veterinary consultancy. No signs of infection were observed. All animals were allowed a recovery time of at least 11 days before infusion.

Infusion

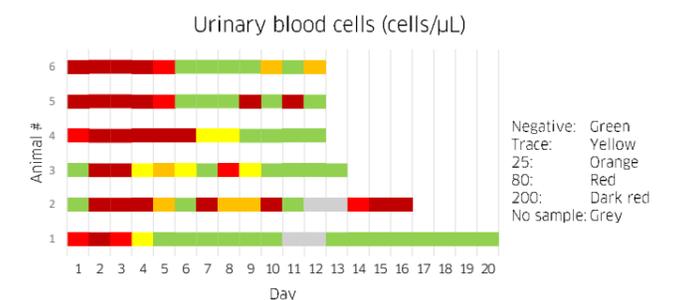
Following the recovery period, a catheter channel was connected to an ambulatory pump and a 5-day infusion protocol of saline was initiated in three animals. Another three animals were infused with a test substance (and data for these infusions are not reported here). The infusion rate was set to approximately 14 mL/h. During this period, no abnormal clinical signs were observed.

Urine analysis

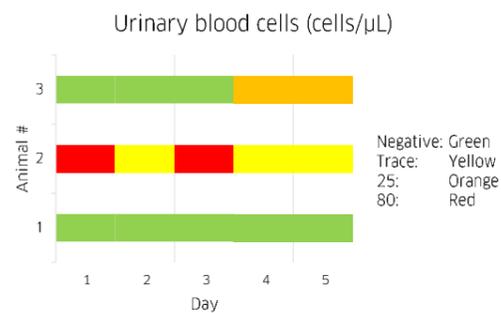
Urine was collected from all animals. The first sample (Day 1) was collected with a syringe during surgery upon exposure of the bladder. Hereafter samples were collected daily through the catheter for additional 11-19 days. Urine was analysed by Clinitek Advantus (urinary chemistry) and Cobas 6000 (ions).

Levels of sodium, potassium, calcium, chloride, and phosphate were all considered to be within our historical control data during the recovery period as well as the 5-day infusion period. The quantitative leucocyte counts were negative (limit of detection is 15 cells/µL) and was further investigated using microscopy.

Following surgery, all animals had high positive urinary blood cell counts (Graph 1) of 200 cells/µL during the first 4 days. Blood cell counts were negative in 3-4 animals at any day from Day 6 and onwards. One individual showed negative counts



Graph 1
Urinary blood cell counts (cells/µL) during the post-surgical recovery period in 6 minipigs. Recovery periods varied between 11 and 19 days.

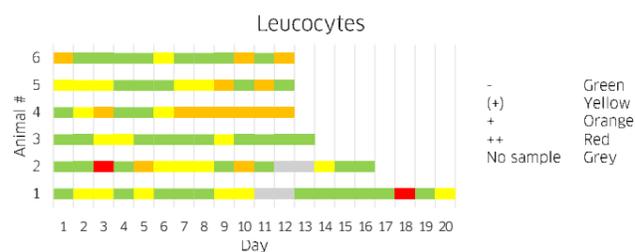


Graph 2
Urinary blood cell counts (cells/μL) during the 5-day saline infusion period in 3 minipigs.

throughout this period while intermittent occurrences of very low (i.e. trace amounts) to single episodes of high level of blood cells were seen in the other individuals. During the 5-day period of saline infusion, the blood cell counts were negative or with trace amounts except for two occurrences of 25 and 80 cells/μL (Graph 2).



Picture 4
One channel of the catheter is connected to an ambulatory infusion pump, which allows for 24-hour infusion. Another channel can be used for sampling of urine.



Graph 3
Semi-quantitative urinary leucocytes during the post-surgical recovery period in 6 minipigs. Recovery periods varied between 11 and 19 days.

The largest number of leucocytes (Graph 3) was present 2 days post-surgery (Day 3) and on Day 5 the levels were identical to levels at Day 1 when urine was sampled by syringe. A slight increase was seen in the number of animals positive for leucocytes for the remaining recovery period. During the 5-day infusion period leucocyte levels remained low (not shown).

Conclusion

A surgical model in the in Göttingen minipig has been established that allows instillation/infusion into the bladder by a 3-way Foley catheter connected to a pump placed on the back of the animal. Urine can be sampled from a catheter channel not used for infusion. The model can be established in both sexes as the catheter is placed in the flank and abdominal wall and is fixed directly in the bladder, which obviates the need for transurethral access. The model proved to be feasible with a post-surgical recovery period of 10 days. A 5 day infusion period, as evaluated from the minor clinical signs and urine analysis did not reveal any significant bleeding or inflammation.

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Naïve plasma levels of Neurofilament Light Chain (NF-L) in different age groups of male and female Göttingen Minipigs

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Genetically modified animal models are a pivotal tool to study human neurodegenerative diseases, e.g. Alzheimer's disease. Considering that the early phases of neurodegenerative diseases often are asymptomatic, an understanding of biomarkers that enable monitoring of disease progression becomes crucial. In this regard, it was suggested that large animal models might offer better translatability to humans than rodent models (Eaton & Wishart, 2017).

Introduction

Over the last few years, genetically modified Göttingen Minipigs have been created to mimic neurodegenerative diseases (Holm, Alstrup, & Luo, 2016), including Alzheimer's disease (Jakobsen et al., 2016; Kragh et al., 2009). However, to understand the disease development and progression in such genetically modified animals, a baseline characterization of relevant biomarkers becomes necessary in order to timely correlate the

phenotype of wildtype animals with the genetically modified conspecifics.

In the past years, Neurofilament Light Chain (NF-L) was established as a biomarker of axonal integrity that can be readily measured in serum, plasma or cerebrospinal fluid, thus enabling longitudinal monitoring of individual animals. Increases in NF-L have been linked to neurodegenerative



Picture 1
Blood sampling of a sling trained Göttingen Minipig.

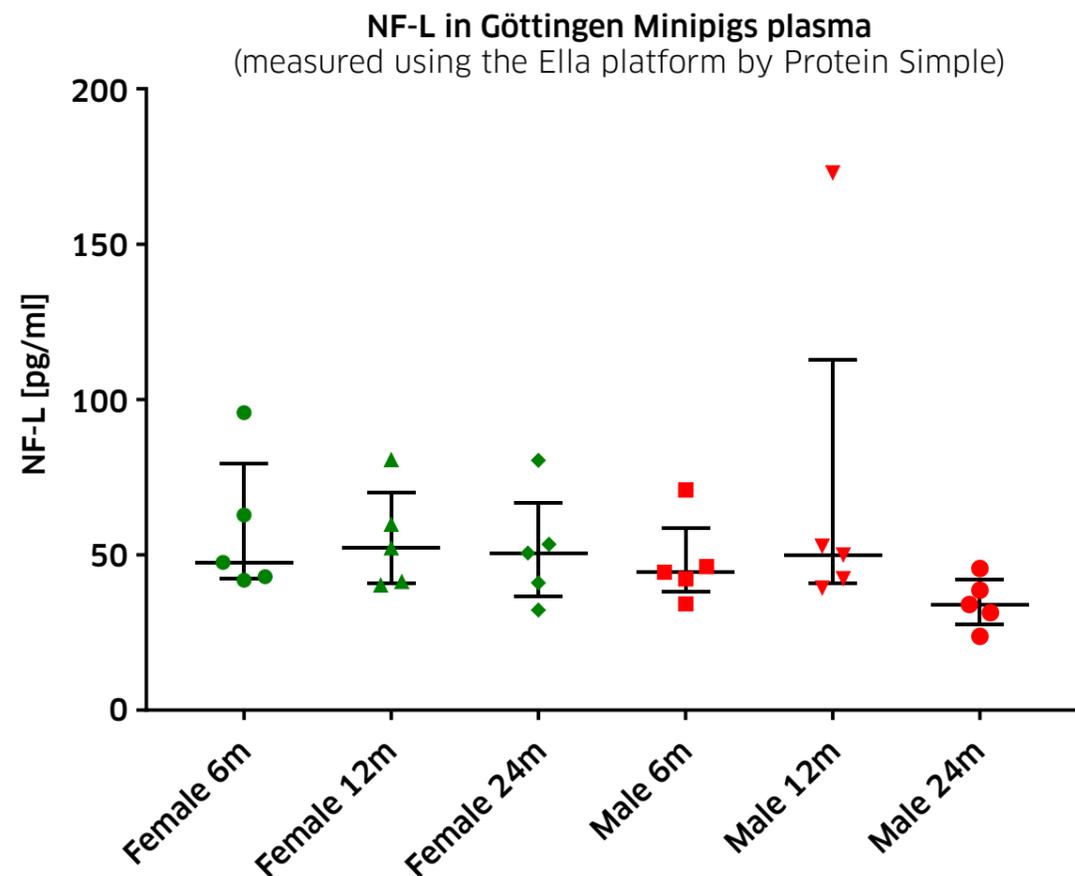


Figure 1
NF-L levels in healthy male and female Göttingen Minipigs at 6, 12 and 24 months (n=5).

diseases in e.g. human Alzheimer's disease (Mattsson, Cullen, Andreasson, Zetterberg, & Blennow, 2019), and have also been observed in transgenic murine models (Bacioglu et al., 2016). Here, we describe the naïve NF-L levels assessed in plasma of wildtype Göttingen Minipigs of each gender and at various age groups.

Methods

To evaluate baseline levels of NF-L and to investigate potential age-related changes, blood was collected from healthy, wildtype Göttingen Minipigs of each gender at the ages of 6, 12 and 24 months (n=5 per age group and sex). The blood samples were collected in sling trained non-anesthetized/non-sedated animals, as anesthesia might impact NF-L levels (Evered, Silbert, Scott, Zetterberg, & Blennow, 2018). The blood samples were processed to plasma, and immediately frozen on dry ice and stored at -80 degrees. The plasma was analyzed for NF-L levels using the Simple Plex NF-L assay according to manufacturer's instructions (ProteinSimple; LLOQ = 2.7 pg/ml; ULOQ = 10 290 pg/ml).

Results

Plasma NF-L levels were comparable between male and female Göttingen Minipigs and no significant age-related differences could be observed (Figure 1). Mean levels were 58.2 pg/ mL

(females) and 47.6 (males) in the 6 months old minipigs, 54.8 pg/mL (females) and 71.5 pg/mL (males) in the 12 months old minipigs and 51.5 pg/mL (females) and 34.7 pg/mL (males) in the 24 months old minipigs. One male animal in the 12 months age group showed strongly increased NF-L levels (173 pg/mL). However, there were no clinical abnormalities observed in this animal, except for a slightly increased heart rate during sampling. Since there were no differences in the sampling procedure compared to the other animals, the reason for the high NF-L levels in this animal could not be determined.

Discussion

The NF-L levels were comparable within animals of the same age group, and across age groups and sexes. It might be possible that an age-related increase in NF-L levels can be observed in healthy animals older than two years. However, these results make it probable that an increase in NF-L levels in genetically modified Göttingen Minipigs younger than 24 months could be potentially related to the neurodegenerative pathology.



Picture 2
Group-housed male Göttingen Minipigs at 12 months of age.

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ACKNOWLEDGEMENTS

The authors could like to thank especially Animal Welfare Technician, Carina Christoffersen and all animal care takers at Ellegaard Göttingen Minipigs A/S being engaged and highly involved in the training and sampling of the Göttingen Minipigs being used in this study.

Working with Göttingen Minipigs

How did you begin your career with research animals?

In my PhD I addressed the issue "Refinement of non-invasive stress and pain assessment in laboratory animals". Because it is difficult to observe stress and pain in mice, it is often presumed that they don't experience any. In the thesis this assumption was proved wrong, and measures how to identify levels of stress and pain and how to best relieve these were assessed.

Which areas within animal research do you believe it is particularly important to focus on?

Research animals play a crucial role within biomedical research. But accommodating their needs and well-being both before, during and after a study is essential - both considering ethics and the validity of data. It goes without saying that stressed animals deliver distorted results, and such data are not comparable to humans who do not experience the same level of stress during treatment. The biases caused by pain and stress are often neglected.

Is it realistic to believe that research animals can always be removed or relieved from stress?

In many cases, yes, it is realistic. However, some studies of course rely on a defined level of stress e.g. animal models of some psychiatric diseases - but it should always be a priority to refine the procedures and methods. I believe in and support the use of animals in research, as long as you can defend the purpose of the study compared to the level of suffering imposed on the animal. It is always a matter of weighing the amount of suffering to the purpose, and researchers are obligated to ensure that no animals suffer unnecessarily. Definition of humane endpoints and a close dialog with laboratory animal veterinarians and animal caretakers working with the animals are key in this process.

About the Science Department

The Science Department at Ellegaard Göttingen Minipigs consists of skilled professionals with specific qualifications within each of their areas. They work closely with the team in the research barrier and includes a Laboratory Animal Veterinarian, an Animal Welfare Technician, and two Laboratory Technicians.

Key functions of the science team include supporting customers with study design, study execution and surgery, and engaging in knowledge sharing about Göttingen Minipigs. They are specialists in the physiology and nature of Göttingen Minipigs, and teach and instruct customers in how to handle Göttingen Minipigs correctly, and constantly improving the welfare of the minipigs.



In focus

Name Kirsten Rosenmay Jacobsen
Function Head of Scientific Management
Unit Science Department

Education
DVM in Biomedicine, PhD in Experimental Medicine.

Background
After finishing her PhD, Kirsten worked a few years in Singapore at a pharmaceutical company as Senior Scientist. Soon after returning to Denmark she was

employed as Laboratory Animal Veterinarian at Ellegaard Göttingen Minipigs.

Engagements

Kirsten is former board member of the Danish Animal Welfare Society, current member of the national Council for Research Animals under The Danish Animal Experiment Inspectorate, and Ad hoc Specialist for AAALAC International in Europe.



Explain your strong belief in purpose and ethics

As humans we have a refined understanding of moral and ethics, and we are obligated to honour this understanding. In general, I believe that too many animals are used by humans for the wrong purposes. However, I also believe that they play a vital and indisputable role within science and medical research and serve a noble purpose in helping and curing humans and other animals from illness and chronic diseases. When pursuing this purpose, it is therefore crucial that we treat animals as humane as possible and continuously refine all aspects of their use and life.

How did you end up working with Göttingen Minipigs?

After returning from Singapore, I wanted to continue working with disease models, medical development, and a large animal species relevant and comparable to humans. Scientists should always use the most relevant animal species for their studies, and pigs have a very high translatability to humans. Furthermore, compared to other non-rodent animal models,

Göttingen Minipigs are very easy to socialise. They are curious and have a well-balanced temper, enabling a teamwork between the minipig and scientist during research, and making it easy to facilitate physical surroundings that accommodate their natural needs in a stable environment.

How can you make a difference in scientific research?

Göttingen Minipigs as a research model have a high construct validity, and data obtained from these animals are very translatable to humans and the human conditions. I want to support preclinical research and contribute to the development of safe and effective treatments, and I can do that by promoting Göttingen Minipigs as an animal model.

You are a member of the national Council for Research Animals under The Danish Animal Experiment Inspectorate and Ad hoc Specialist for AAALAC International. Why is that a priority to you?

Engaging in these activities is one way for me to advocate for and practice my passion about animal welfare within biomedical research. As Ad hoc Specialist for AAALAC I also get the opportunity to influence scientific research involving research animals on a European level.

What are your aspirations for the science team at Ellegaard Göttingen Minipigs?

With Göttingen Minipigs as an established animal model, we help scientists conduct proof-of-concept studies and create scientific set-ups accommodating scientists' needs. Creating the best possible framework for our customers' research results will continue to be one of our highest priorities.



Outsourcing of pre-experimental study parts in preclinical cardiovascular studies

Göttingen Minipigs are increasingly selected for all aspects of pharmaceutical research and are fully recognized as a reliable and established animal model by all regulatory authorities worldwide. This section aims at providing an insight into the wide use of Göttingen Minipigs within biological research. If you know of an interesting study, you are welcome to reach out.

Insight provided by:

Thomas Mondritzki, Head of Heart and Vascular Diseases IV | Bayer AG, Germany

What is the project about?

This project describes the evaluation of outsourcing of pre-experimental study parts in preclinical cardiovascular large animal studies. In order to characterize new test substances in telemetered animals, it is of great importance that these animals are pre-trained for individual procedures. On the one hand, this allows some individual procedures (e.g. treadmill analysis); on the other hand, high-quality data (without stress artefacts) can be collected. Adequate training of animals require a lot of time during which the animals, and thus also stables, are not used for experimental studies. This ties up resources and reduces the productivity of individual laboratories. The same applies to telemetry sensor implantations: After sensor implantation, a wound healing phase of several weeks is necessary, during which the animals and stables are not usable for experimental studies. Outsourcing these activities could therefore significantly increase laboratory productivity.

What is the purpose of the project?

During a site-visit at Ellegaard Göttingen Minipigs in Denmark, March 2019, I realized that they employ experts who are able to perform such complex surgical interventions, like sensor implantations, and that they have lots of experience in animal

training. Furthermore, they have the facilities, equipment and logistics to support such studies. The idea was born that we could transfer parts of these studies (specially the pig training and sensor implantation incl. woundhealing phase) to Ellegaard Göttingen Minipigs.

We initiated a 'Proof-of-concept study' to evaluate the benefit of outsourcing animal training activities and telemetry implantations in minipigs to Ellegaard Göttingen Minipigs.

This study includes a technology transfer from Bayer to Ellegaard Göttingen Minipigs, and Bayer employees should train experts from Ellegaard Göttingen Minipigs in sensor implantations.

Regarding animal training, a desirable outcome of the animal training should be:

- A) feasibility of blood collection in awake animals
- B) possibility of oral, intravenous and subcutaneous applications
- C) execution of treadmill exercise tests with a pre-defined protocol

All of the above without excessively stressing the minipigs.

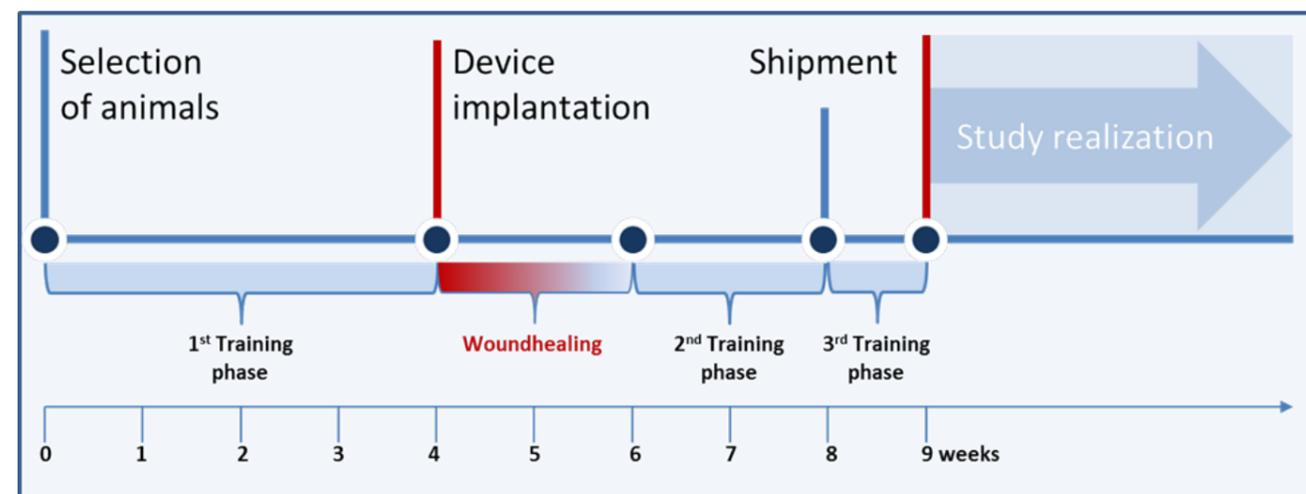


Figure 1
Study protocol.



Picture 1
Surgical room for sensor implantation.
Source: Ellegaard Göttingen Minipigs A/S

Why is it important?

Laboratories performing large animal studies are highly specialized and need to know their models in detail. However, these laboratories are facing increasingly complex challenges. Preclinical cardiovascular animal studies are more and more complex, due to heterogeneous nature of the disease, the presence of comorbidities, and lack of appropriate outcome parameters. These labs must also be flexible with regard to changes in the research topics and always be able to adapt their models to new requirements. This binds a lot of resources. In order to focus on the sensitive and decision-relevant parts of the study. Other parts of the study should be outsourced to partners who are experts in the field.

What makes this project particularly interesting?

The translation of early research findings via animal models into the human disease situation in drug development is still limited. Several reasons for this lack of predictivity are discussed in the literature. Drawbacks frequently relate to the use of anesthetics in animal research. It is well known, that almost all available anesthetics can influence hemodynamics and other physiological parameter leading to a potential bias in the study readouts. This study could be a model for further collaborations. Laboratories that have spared the effort of implementing telemetry studies, could find an option to realize such studies and with this to improve the predictive value of their studies.

How do you recommend going about species selection?

In the cardiovascular field, studies should not rely on single models or single species. Species differences exist in autonomic function, coronary anatomy and response to drugs. Because of these and other differences, several species should always be considered for a widening investigation. The choice of the model should always depend on the individual research question, whereas pig models play an important role with regard to similarities in cardiovascular physiology to humans.



Picture 2
Animal treadmill training.
Source: Ellegaard Göttingen Minipigs A/S

Spotlights

Göttingen Minipigs Symposium 2020 hosted by Marshall BioResources

This year the Göttingen Minipigs Symposium, hosted by Marshall BioResources, has transitioned into an online event. Presentations will be conducted in a series of webinars during this autumn and we welcome professionals around the world to participate in this event.

The focus for this year's symposium will be on the use of Göttingen Minipigs in biodefense research, offering the opportunity to join many interesting online presentations.

For scientific program and registration, follow updates at marshallbio.com/gms2020.



Study confirms the breed integrity of Göttingen Minipigs

The paper "Assessing breed integrity of Göttingen Minipigs" confirms that the breed integrity of Göttingen Minipigs remains intact across continents due to our unique breeding program. This allows scientists to compare results directly and exchange knowledge unconcerned whether studies are using Göttingen Minipigs bred in Europe, the United States, or Japan.

Visit minipigs.dk to read the full paper.



Underweight piglet survives

Piglets from Göttingen Minipigs usually weigh around 450-550 g. at birth, so when a piglet was born weighing only 190 g. and still managed to survive, it is worth noticing.

To ensure that the piglet assimilated enough antibodies during the first 24 hours of life, it was hand fed with colostrum in a syringe and received extra heat and caring during the first few weeks after farrowing. "When newborn piglets are this small and weak, their chances of making it are slim. Weaning was when we would see if the effort had paid off, and luckily the tiny piglet turned out just as strong and healthy as the rest of the litter" Tania Panfilova, Animal Caretaker at Ellegaard Göttingen Minipigs, explains.

Comprehensive MR and CT imaging atlas

A comprehensive imaging atlas now offers the unique opportunity to follow the anatomical evolution of Göttingen Minipigs. The images are made from high-resolution CT- and MRI-scans and fully support the 3R principles.

The atlas is available to anyone interested in the physiology of Göttingen Minipigs. Get more information about the atlas and learn how to get access to the scans from our website: minipigs.dk/imaging-atlas-info

In picture: CT scan showing frontal and distal segmentation of bone structure and cardiovascular system.



Health Monitoring Report: June 2020

Every 6 months the health monitoring report, based on FELASA recommendations, is published. "We are very pleased to confirm, that the report from June 2020 documents that there are no changes in the overall health status at our facility", says Kirsten Rosenmay Jacobsen, Head of Scientific Management at Ellegaard Göttingen Minipigs.

Download the full report from minipigs.dk/goettingen-minipigs/health-status.

Genetically altered Göttingen Minipigs Models

In recent years, the numbers of genetically altered Göttingen Minipigs models have increased, as advanced genetic techniques simplify the generation of animals with precisely tailored modifications. These modifications are designed to replicate genetic alterations responsible for human diseases and enable the development of safe and effective medicines improving the quality of life for affected patients. For this, genetically altered Göttingen Minipigs are valuable large animal disease models.

Using Göttingen Minipigs as background strain for the creation of genetically altered minipig models will be commercially available during 2021. Contact ellegaard@minipigs.dk for more information.

In picture: The first successful litter of genetically altered Göttingen Minipigs piglets.





The 14th Minipig Research Forum

We enable development of safer and more effective medicines

At Ellegaard Göttingen Minipigs we are all for sharing and believe that openness creates trust, enriches and clears the path for new opportunities. **We share knowledge** about Göttingen Minipigs for biomedical research, both our own knowledge but also learnings from scientists around the world. **We create fora** for networking and knowledge sharing amongst scientists. **We support scientific research** through our Research Foundation. **We educate** through webinars and practical courses.

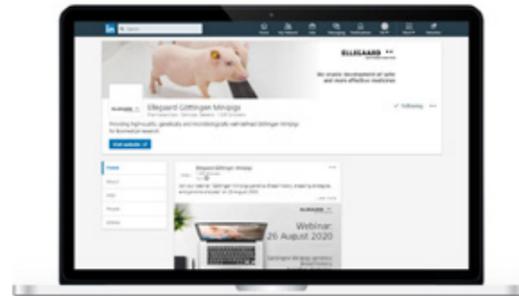
Subscribe to news and invitations

Receive invitations to webinars and scientific meetings, new Göttingen Minipigs Magazine publications and other news directly in your inbox, by subscribing to news from Ellegaard Göttingen Minipigs: minipigs.dk/sign-up-for-news

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Add Ellegaard Göttingen Minipigs to your feed and be notified of:

- Scientific meetings
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- Publications and research results
- Health status incl. Health Monitoring Reports, health screenings, accreditations etc.
- Project call-outs from Ellegaard Göttingen Minipigs Research Foundation



Facility of Ellegaard Göttingen Minipigs A/S

New dates and venue | Mark your calendar:

29 September - 1 October 2021
at Ellegaard Göttingen Minipigs A/S in Dalmose, Denmark

REMIND ME, WHAT IS MRF ABOUT?

The Minipig Research Forum is a unique opportunity for Göttingen Minipigs users to meet, discuss and share knowledge and experiences within all areas of minipig use related to biomedical research. Mark your calendar for this 3-day conference packed with scientific lectures, poster presentations and the opportunity of networking with minipig users from all over the world.



Stay updated on the full scientific program and for registration opening at the MRF website and the [LinkedIn group](https://www.linkedin.com/groups/1234567890123456789/).

ABOUT THE CHOICE OF DATE

The MRF meeting will be held in continuation of EUROTOX 2021, that will be held in the city centre of Copenhagen, thus potentially enabling you to participate in both events with less traveling activity. In addition to this obvious time and cost reduction, you will also leave a significantly smaller carbon footprint. Of course, you are welcome to attend the MRF without attending EUROTOX 2021, as the two events in no ways are co-hosted or directly linked.

ABOUT THE LOCATION

The event will be hosted in Ellegaard Göttingen Minipigs' brand new conference room at their site, located only 1½ hour outside the city of Copenhagen and Copenhagen Airport, in the beautiful Danish countryside. During the MRF you will get a unique opportunity to visit the breeding and research facilities of Ellegaard Göttingen Minipigs. Of course, as usual during an MRF meeting, you will be able to network with minipig users from all over the world; a highly valued part of the meeting and a very important reason why we refrain from arranging a virtual MRF meeting.

Attend webinars

If you are interested in specific topics, or you would like to share your knowledge or experience with Göttingen Minipigs in one of our webinars, please contact us on events@minipigs.dk

Topic	Date	Guest speaker	Register
Optical coherence tomography (OCT) for ocular safety assessment in Göttingen Minipigs	29 September 2020 2 pm CEST*	Nora Denk F. Hoffmann-La Roche, Switzerland	bit.ly/EGMweb200929
Inhalation studies in minipigs	20 October 2020 10 am CEST*	Simon Moore Covance, United Kingdom	bit.ly/EGMweb201020
Minipigs in translational immunosafety assessment	5 November 2020 10 am CET**	Tina Rubic-Schneider & Michael Kammüller Novartis, Switzerland	bit.ly/EGMweb201105
Development of a urinary tract infection animal model in Göttingen Minipigs	3 December 2020 15 pm CET**	Jeffrey Fernandez & Jessica Henn Janssen Research & Development, USA	bit.ly/EGMweb201203

* Central European Summer Time

** Central European Time

The MRF is one of my favorite conferences: Not too big, great people and networking

Good mixture of science, practical topics, animal welfare and networking/discussions

My first MRF: loved it totally and found everything to be very well organized



The MRF is a non-profit organization with more than 500 members worldwide working with minipigs in industry, academia and regulatory bodies. Participation in the annual MRF conference requires membership (free of charge). Read more and apply for membership at www.minipigresearchforum.org

Ellegaard Göttingen Minipigs Research Foundation: Donations in 2020

Supporting and participating in scientific research activities involving Göttingen Minipigs is a fundamental part of the business foundation at Ellegaard Göttingen Minipigs. Therefore, the Ellegaard Göttingen Minipigs Research Foundation was founded in 2016 to actively support these activities, and holds the main objective of providing financial funding for scientific research based around Göttingen Minipigs.

Criteria for funding

In 2020, the Ellegaard Göttingen Minipigs Research Foundation grants in total up to €50,000 to support activities that aim at characterizing Göttingen Minipigs or promoting the development of Göttingen Minipigs based disease models. In addition, projects that intend to improve animal welfare, focus on the 3Rs, and/or optimize handling or research techniques as well as educational and communication activities in relation to Göttingen Minipigs use may receive funding, as long as the project will generate significant background data and/or ensure knowledge dissemination and promote the use of the Göttingen Minipigs in scientific research.

Supported projects in 2020

Over the last months, the Ellegaard Göttingen Minipigs Research Foundation has supported three exciting and very diverse research projects:

- A study assessing the potential of a new, innovative treatment of patients suffering from COVID-19. The study is briefly mentioned as part of the scientific article on p. 6-7 in this edition of the Göttingen Minipigs Magazine.
- The development of a Göttingen Minipigs based acute bleeding model evaluating a new treatment for traumatic and often fatal bleedings.
- A project aiming at establishing non-invasive cardiorespiratory monitoring in juvenile Göttingen Minipigs.

The outcome of all three funded research project will be presented in later editions of the Göttingen Minipigs Magazine.

Apply for funding

To learn more about the Ellegaard Göttingen Minipigs Research Foundation or apply for funding, please visit minipigs.dk or contact Head of Scientific Management at Ellegaard Göttingen Minipigs, Kirsten Rosenmay Jacobsen on krj@minipigs.dk.



ENVIRONMENTAL ENRICHMENT FOR GÖTTINGEN MINIPIGS

Carina Christoffersen & Kirsten Rosenmay Jacobsen | Ellegaard Göttingen Minipigs A/S | Denmark

ABSTRACT

Environmental enrichment is an important part of refined housing and care for laboratory animals. It has wide-ranging physiological and behavioral effects and can be particularly effective to stimulate natural behaviors and reduce the incidence and/or severity of undesirable or abnormal behaviors. An ideal enrichment program is attractive to the animals and results in sufficiently sustained interest. Hence, it is important to have a variety of suitable enrichment items to rotate between the animals and to stimulate different aspects of their behavioral profile. Here we present some of the enrichment items that we find beneficial to use for Göttingen Minipigs in a research and barrier environment.

BEHAVIOR

Göttingen Minipigs (GM) possess a great explorative and investigative motivation, and they will spend much of their awake time rooting and exploring the surroundings. Natural products such as straw and hay prompt and stimulate these natural behaviors, and can be used in a variety of ways to maintain novelty and remaining interest. The use of racks or dispensers increase the time engaged in these behaviors. Furthermore long fibered hay placed in racks increases nesting behavior the day before farrowing for pregnant sows. Homemade devices such as ice cubes with or without straw or hay and flavor are a good supplement to engage minipigs in short term active behaviors. Straw and hay can be irradiated to eliminate a potential microbiological risk.



OCCUPATIONAL ENRICHMENT

Many types of commercial enrichment devices can be used to stimulate GM. Items that stimulate multiple behaviors are more likely to engage the minipigs for a longer time e.g. toys that dispense food items or provide attractive sensory stimulation.

The "Porcichev" (www.porcichev.com) enrichment device stimulates manipulation and biting, and is impregnated with strong scents which are very appealing to the pig: the more the pig bites and plays with the toy, the more scent is released.

The "Easyfix Luna" (www.easyfix.com) enrichment device have 12 legs enabling multiple pigs to interact, manipulate, bite and play at the same time. The devices come in different sizes to match the age of the pigs.

For older animals and especially boars, biting and manipulation can be stimulated by using wood bricks (www.tapvei.com). Sexually mature boars further benefit from large ball (www.schippers.dk)—especially if they are single housed. The boars will use the ball for manipulation, pushing it around and to perform their natural mounting behavior.

As conventional swine, GM are highly food motivated. Treats can be used during socialization, and as reward when training the minipigs to participate in husbandry or study related procedures. Furthermore, long duration treats can be beneficial during mixing of animals and reduce the level of fighting. The Bunny (www.bio-serv.com) with different fruit flavor can be used even in a barrier facility where fresh fruits or similar are not possible to bring in or include in a GLP study.

Depending on the pen design, it can be beneficial to hang enrichment devices in a chain. Furthermore, it is imperative for the use of any occupational enrichment device that it is removed from the pen and reintroduced at intervals to avoid boredom.

ENRICHMENT ROOM

Dedicated play pens/enrichment rooms can be implemented to stimulate play and active behavior and it can be adapted to any facility and group of animals. An enrichment room is an effective way to increase behavioral diversity, and it is our experience that the room and its devices are of continuous interest.

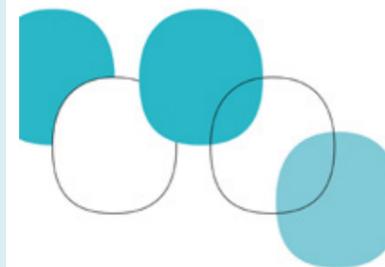
Minipigs aged 4 to 15 weeks benefit from 20-30 minutes play time on a weekly basis. Different items can be included—only the imagination sets the limits—e.g. balls of various sizes, water pools, tunnels and even non-enrichment devices can be included and will be used both during play sessions and to habituate the animals to the items (e.g. a transport cage). We believe that an enrichment room improves the pigs' adaptability to novelty and it may reduce fear in novel situations. Furthermore, the room enhances social interaction – both between the pigs and between the pigs and the staff.



CONCLUSION

As for all animals species, the captive environment should allow the animals to perform their natural species specific behaviors. For Göttingen Minipigs, rooting, manipulation and social interactions can be stimulated by various enrichment devices and items. In addition to improving the home-pen environment, multiple enrichment items can be used in a dedicated enrichment room to allow the animals to engage in new activities and complex social behavioral events for a dedicated time.

For more information please contact: cc@minipigs.dk / krj@minipigs.dk or visit www.minipigs.dk



ELLEGAARD ••
GÖTTINGEN MINIPIGS

New publications on Göttingen Minipigs

Ellegaard Göttingen Minipigs gives high priority to collaborative projects that aim to better characterize and validate Göttingen Minipigs as a translational animal model and which facilitate and refine the use of Göttingen Minipigs in research projects and safety testing. Do you have an idea for such a collaborative project? Please contact us.

Cirera S, Tasöz E, Jacobsen MJ, et al.

The expression signatures in liver and adipose tissue from obese Göttingen Minipigs reveal a predisposition for healthy fat accumulation

Nutr Diabetes. 2020 Mar 23;10(1):9. doi: 10.1038/s41387-020-0112-y

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Brain Struct Funct. 2020 Apr;225(3):1055-1071. doi: 10.1007/s00429-020-02058-x. [Epub ahead of print]

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Cutan Ocul Toxicol. 2020 Jun;39(2):143-157.

doi: 10.1080/15569527.2020.1751183. [Epub ahead of print]

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Circ Res. 2020 Apr 24;126(9):1297-1319. doi: 10.1161/CIRCRESAHA.120.315930. [Epub ahead of print]

<https://pubmed.ncbi.nlm.nih.gov/32324497/>

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<https://pubmed.ncbi.nlm.nih.gov/32477113/>

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Mol Imaging Biol. 2020 Jun 8. doi: 10.1007/s11307-020-01506-8. [Epub ahead of print]

<https://pubmed.ncbi.nlm.nih.gov/32514885/>

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<https://pubmed.ncbi.nlm.nih.gov/32616907/>

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Cellular, mitochondrial and molecular alterations associate with early left ventricular diastolic dysfunction in a porcine model of diabetic metabolic derangement

Sci Rep. 2020 Aug 6;10(1):13173. doi: 10.1038/s41598-020-68637-4.

<https://pubmed.ncbi.nlm.nih.gov/32764569/>

Basu D, Bornfeldt KE

Hypertriglyceridemia and Atherosclerosis: Using Human Research to Guide Mechanistic Studies in Animal Models

Front Endocrinol (Lausanne). 2020 Aug 6;11:504. doi: 10.3389/fendo.2020.00504. eCollection 2020.

<https://pubmed.ncbi.nlm.nih.gov/32849290/>

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Metabolomics in Radiation Biodosimetry: Current Approaches and Advances

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<https://pubmed.ncbi.nlm.nih.gov/32796693/>

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Where to meet us in 2020



CONGRESS / SYMPOSIUM	DATE	LOCATION
SOT and ToxExpo	Cancelled	Anaheim, California, USA
AST Congress	Cancelled	Edinburgh, Scotland
Minipig Research Forum (MRF)	Cancelled	Lisbon, Portugal
Göttingen Minipigs Symposium	Webinar series	Online event
AFLAS	Cancelled	Chiang-Mai, Thailand
World Congress (WC11)	Cancelled	Maastricht, Holland
EUROTOX	Cancelled	Copenhagen, Denmark
GV-SOLAS	16-18 September	Online event
Janssen Juvenile Toxicology Symposium	Cancelled	Beerse, Belgium
3R's Research and Progress	19-20 November	Hyderabad, India
LASACON	21-22 November	Hyderabad, India
CALAS	Cancelled	China
AFSTAL	1-3 December	Marseille, France

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