No. 59 | Winter 2020

Göttingen Minipigs
MAGAZINE

ELLEGAARD
GÖTTINGEN MINIPIGS

Göttingen Minipigs Magazine | 59 | 2020

Dear reader

2020 is coming to an end. A year that in part will be remembered for the tragic incident of the Covid-19 pandemic. We have all learned a lot from the past 10 months, as we have had to adapt to a new reality - both personally, professionally, and as communities. Though humans are known for their ability to adapt to any conditions, I think many are impressed just how well we have managed to keep the wheels turning on so many levels. Change was needed, but many of these changes we can benefit from even in a post-pandemic world.

Despite facing global lockdowns, we have experienced an amazing level of activity at Ellegaard Göttingen Minipigs and a persistent interest in our Göttingen Minipigs. We have delivered minipigs and biological material, conducted research projects at our research facility, and advised scientists in their studies every day throughout 2020. Thank you all for your support and enthusiasm.

Sadly, but necessarily, most scheduled conferences were cancelled this year. This instead speeded up other knowledge-sharing platforms and manifested itself particularly in virtual meetings and webinars, and we are becoming increasingly used to seeing each other through a webcam. This virtual turnaround has proved efficient for some purposes, and I am proud and honored that our webinar series is a successful and popular example of this. They will of course continue moving forward.

At Ellegaard Göttingen Minipigs we now look ahead and hope for a prosperous 2021 as we are excited about new initiatives, new events (both online and offline) and making new connections entering new markets. I hope you will look out for our announcements as they are shared throughout 2021.

Last but not least: Let us keep staying together by staying apart for a little while longer, so we can make 2021 a successful and healthy year.

Merry Christmas and Happy New Year,

Lars Friis Mikkelsen, CEO
Ellegaard Göttingen Minipigs A/S

CONTACT

Ellegaard Göttingen Minipigs A/S

Soroe Landevej 302
4261 Dalmose
Denmark

+45 5818 5818

ellegaard@minipigs.dk

www.minipigs.dk

CONTENTS

10 years after RETHINK saw the light of day

Exactly 10 years ago, the use of Göttingen Minipigs in biomedical research was kickstarted by the publication of the RETHINK project in a special issue of the Journal of Pharmacological and Toxicological Methods (J Pharmacol Toxicol Methods 62, Nov-Dec 2010). This is a milestone that deserves to be celebrated, as Göttingen Minipigs has since drawn much attention and is an important large animal species in the preclinical safety and toxicology assessment of new medicines and treatments.

The objective of the RETHINK project was to assess the potential impact of toxicity testing in the minipig as an alternative approach in regulatory toxicity testing, which could also contribute to the replacement, refinement, and reduction of animal testing in line with the Principles of the 3Rs. Several Expert Working Groups were assembled to review five different areas relating to the use of minipigs in regulatory safety testing:

- Ethical issues
- Welfare and animal care
- Development of new medicines and chemicals
- Safety testing issues
- Emerging technologies in safety testing

When the conclusions and recommendations of the Expert Working Groups were presented, they all concluded that there are no specific areas where restrictions to the use of minipigs in toxicology are required for welfare reasons.

The RETHINK project

The following articles were published in 2010 in a special issue of the Journal of Pharmacological and Toxicological Methods (J Pharmacol Toxicol Methods 62, Nov-Dec 2010):

The RETHINK project: Minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment
By R. Forster, G. Bode, L. Ellegaard, J. W. can der Laan, and Steering Group of the RETHINK Project

Ethical implications of using the minipig in regulatory toxicology studies
By J. Webster, P. Bollen, H. Grimm, M. Jennings, and under the auspices of the Steering Group of the RETHINK Project

Welfare of the minipig with special reference to use in regulatory toxicology studies
By L. Ellegaard, A. Cunningham, S. Edwards, N. Grand, T. Nevalainen, M. Prescott, T. Schuurman, and under the auspices of the Steering Group of the RETHINK Project

Regulatory acceptability of the minipig in the development of pharmaceuticals, chemicals and other products
By J. W. van der Laan, J. Brightwell, P. McAnulty, J. Ratky, C. Stark, and under the auspices of the Steering Group of the RETHINK Project

The utility of the minipig as an animal model in regulatory toxicology
By G. Bode, P. Clausing, F. Gervais, J. Loegsted, J. Luft, V. Nogues, J. Sims, and under the auspices of the Steering Group of the RETHINK Project

Göttingen Minipigs Magazine | 59 | 2020

Today, the minipig model is generally accepted by regulatory authorities, provided it is adequately justified. The minipig has become an interesting and highly relevant model for safety testing since there are numerous anatomical, physiological, genetic, and biochemical similarities to humans. In addition, many features of the minipig make it a practical and flexible model for safety testing.

The use of minipigs in development of products does not bring any financial penalty in terms of the costs of testing. Benefits referring to the 3Rs can be identified in terms of life-cycle analysis of the use of minipigs compared to dogs and non-human primates. Finally, the minipig, unlike the dog, is well positioned to take advantage of genomics and gene manipulation technologies. Further specific recommendations for supplementary research were presented, which adds to the 3R benefits and arguments.

To deploy the minipig to the best advantage, clear information is needed about the predictivity of the minipig for human toxicities and focused action to define the potential role of the minipig in testing of biologics.

In the 10 years that have passed, many publications have highlighted Göttingen Minipigs as a superior animal model within many disease areas and as an equal species with high translatable value in preclinical safety assessment. Two specific references that deserve mentioning are the “The minipig in biomedical research”, a book edited by Peter A. McAnulty and co-workers, published by CRC Press in 2012; and the special issue “Swine in Translational Research and Drug Development” in Toxicologic Pathology, Volume 44, Number 3 from 2016. Both describe in detail the use of minipigs in biomedical research and follow up on previous papers, including the ones that are the outcome of the RETHINK project.

In addition, several hundred papers have been individually published to describe, characterize, validate, and support the use of Göttingen Minipigs, including recent papers describing promising genetically altered disease models.

Enquiries about specific references or the use of Göttingen Minipigs in biomedical research in general can be directed at ellegaard@minipigs.dk.

Genetic management of the Göttingen Minipig population
By H. Simianer and F. Köhn

The minipig as a platform for new technologies in toxicology
By R. Forster, P. Ancian, M. Fredholm, H. Simianer, B. Whitelaw, and under the auspices of the Steering Group of the RETHINK Project

The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals: Conclusions and recommendations
By R. Forster, G. Bode, L. Ellegaard, and J. W. van der Laan

Neonatal and juvenile ocular development in Göttingen Minipigs

By Vanessa Vrolyk[1,2], Julius Haruna[2], and Marie-Odile Benoit-Biancamano[1]

[1]Research Group on Infectious Diseases in Production Animals (GREMIP) and Swine and Poultry Infectious Disease Research Center (CRIPA), Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, Québec, Canada | [2]Charles River Laboratories Montreal ULC, Laval, Quebec, Canada

The use of juvenile animals in preclinical toxicity studies conducted for drug development has generated substantial interest over the last decade.[11,15] Regulatory agencies, such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), are now considering the use of juvenile animals essential in order to perform a proper risk and safety assessment of new xenobiotics intended for the pediatric population.[5,6]

It is known that children or immature animals do not always respond to xenobiotics in the same manner as adults do when it comes to drug efficacy and/or toxicity.[1,3,4,8] The evaluation of tissue samples from juvenile animals brings additional challenges for pathologists involved in preclinical toxicity studies. Indeed, they must not only identify "standard" drug-related effects, but must also detect any developmental abnormality/delay and not misinterpret physiological developmental events as drug-related changes. Thus, an excellent understanding of the normal histology of developing organs in various species is crucial. Yet, as age-matched controls are not always available in juvenile toxicity studies based on the study design, pathologists often rely on published literature to find reference information on the normal histology of structures collected during the postnatal phase of development; however, such data is still limited and incomplete, particularly for Göttingen Minipigs. Comprehensive histological descriptions of immature tissues from juvenile animals become even more critical for organs composed of highly sophisticated and complex structures, such as the eye.

Study objectives and design

This study aimed to 1) characterize the normal postanal histomorphological ocular development in Göttingen Minipigs from birth until adulthood, 2) establish the age timepoints when each structure of the eye reaches histomorphological maturity, i.e. when the histology is similar to that of the adult mature eye, and 3) compare the histology of developing eyes from Göttingen Minipigs with the eyes of age-matched domestic pigs.

To conduct this study, 16 Göttingen Minipigs divided into groups based on age (postnatal day [PND] 1, 7, 14, 21 and 28; 2 months, 3 months and 6 months) were donated by Marshall BioResources, New York, USA. Twenty five (25) age-matched F2 domestic pigs were obtained via the Diagnostic Service of the Faculty of Veterinary Medicine of the Université de Montréal, Quebec, Canada.

For all animals, a thorough histological evaluation of the eyes was performed by an American College of Veterinary Pathologists (ACVP) board certified pathologist using standard hematoxylin and eosin staining, and immunohistochemistry labeling was done to detect ki-67, caspase-3, GFAP, calbindin, synaptophysin and rhodopsin.

What did we learn from this study?

Despite the more advanced ocular developmental stage in neonatal Göttingen Minipigs compared to other commonly used laboratory animals such as rodents and dogs,[16,19] histomorphological signs of immaturity were observed in every structure of the eye of Göttingen Minipigs at birth, and the eyes continued to develop until 6 months of age.

Examples of noteworthy histological and immunolabeling features highlighting the immaturity of the eye of Göttingen Minipigs at birth, and for variable time periods thereafter depending on the structures, are listed in the table above and illustrated in figure 1.

Furthermore, this study was the first to report histomorphological and immunolabeling pattern differences between the area centralis (or visual streak) region of the retina and other retinal regions, in juvenile and adult Göttingen Minipigs. Notably, this study has shown obvious variations in the distribution of a new subset of cone photoreceptors positive for calbindin between the different regions of the retina.

Overall, when compared to the adult mature eye, the eye of Göttingen Minipigs was considered fully developed histologically at 6 months of age. The age timepoints when specific structures reached histomorphological maturity are included in figure 1.

Readers are invited to refer to the published article of this research for the complete results and further details.[18]

Is the postnatal ocular development in Göttingen Minipigs different from that of domestic pigs?

Histologically speaking, the eyes of adult Göttingen Minipigs and domestic pigs are very similar. This study reported few subtle histological and/or immunohistochemical variations between these 2 breeds suggesting that some structures, such as the cornea and lens, would be slightly more developed at birth in domestic pigs compared to Göttingen Minipigs. Nevertheless, every structure of the eye reached histomorphological maturity at the same age timepoints, in both pig breeds.

Göttingen Minipigs Magazine | 59 | 2020
Science

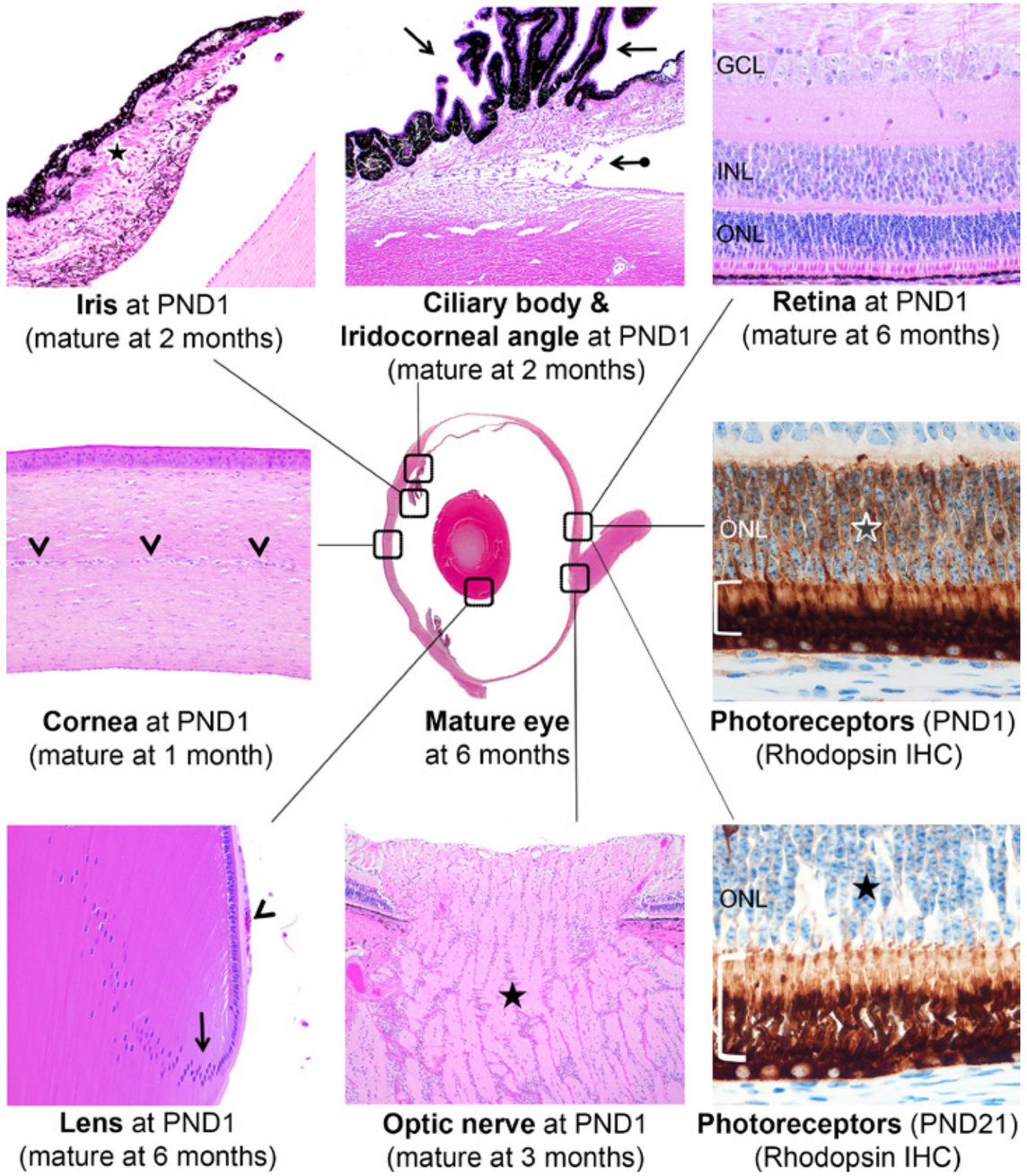


Figure 1

Figure legend

Overview of selected histomorphological and immunohisto-chemical features of the postnatal ocular development in Göttingen minipigs

Cornea: at postnatal day (PND 1), capillaries (arrowheads) are present in the stroma.
Iris: at PND1, the stroma in more cellular and the sphincter muscle (star) is thinner.
Ciliary body: at PND1, ciliary processes (arrows) are shorter and thinner.
Iridocorneal angle: at PND1, the angle (arrow with circle) is narrow and shallow.
Lens: at PND1, the bow region (arrow) is slightly more cellular and capillary remnants (arrowhead) from the tunica vasculosa lentis are visible along the lens capsule.
Retina (top right): at PND1, in the area centralis (visual streak) region, the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL) are composed of more numerous cell layers.
Photoreceptors at PND1 (middle right): Rhodopsin immunolabeling highlighting the shortness of rod photoreceptors (bracket) and the presence of labeling in the ONL (star).
Photoreceptors at PND21 (bottom right): Rhodopsin immunolabeling showing the elongation of rod photoreceptors (bracket) and the loss of labeling in the ONL (star).

Key histological features	Key immunolabeling results
• Vascularization in the corneal stroma • Thin Descemet's membrane in cornea • Narrow and shallow iridocorneal angle (filtration angle) • Short and thin ciliary body processes • Sparse iris and ciliary body muscles • Vascular remnants from the fetal hyaloid vasculature • Markedly thin lens capsule • Nuclear remnants in secondary lens fibers • Increased cellularity in retinal layers • Immature photoreceptor morphology • Increased cellularity in the optic nerve	• Ki67: increased cellular proliferation in almost all developing structures • Caspase-3: apoptosis in the retinal inner nuclear layer and regressing hyaloid vasculature remnants • GFAP: shortness of retinal Müller cell processes • Rhodopsin, synaptophysin and calbindin: shortness and immature morphology of rod and/or cone photoreceptor inner and/or outer segments

Table 1
Selection of key histological and immunolabeling features of the immature eye of Göttingen minipigs observed during the postnatal developmental phase.

Are Göttingen Minipigs a good model for ocular (juvenile) research?

Minipigs are increasingly considered a relevant animal model for ocular research in the scientific field and for preclinical toxicity studies as they share several histological and anatomical similarities with the human eye.[9,10,13,17] Most importantly, minipigs and domestic pigs are particularly relevant for retinal research as the swine retina contains a region called the area centralis (or visual streak) which mimics to some extent the macula in the human eye, a structure associated with sight-threatening conditions in people, such as macular degeneration, which are widely studied.[2,7,17,18] The current study has taught us that the developmental stage of the eye of Göttingen Minipigs at birth, and particularly of the retina, more closely resembles that of neonatal human and non-human primates, compared to other commonly used laboratory animals, such as rodents and dogs, which are born with markedly underdeveloped eyes.[12,14,16,18]

Take-home message

Overall, this study showed that the eyes of Göttingen Minipigs are not fully developed at birth and still undergo histological and immunohistological developmental changes until 6 months of life, when the eyes are considered histomorphologically mature. Compared to other commonly used non-primate laboratory animals, such as rodents and dogs, the developmental stage of the eyes of Göttingen Minipigs at birth more closely resemble that of neonatal human, making the minipig a promising model to study pediatric ocular diseases or for the development of ophthalmic drugs intended for use in children.

Picture 1
Göttingen Minipig at postnatal day 7.

Göttingen Minipigs Magazine | 59 | 2020

ACKNOWLEDGEMENTS

We are thankful to Michelle Salerno and Nicole Navratil from Marshall BioResources, North Rose, New York, for donating all Göttingen minipigs and to the technical staff from Charles River Laboratories Montreal ULC for the eye collection and preparation of histological and immunohistochemical slides.

FUNDING

The study was supported through grants from Mitacs, the Fonds du Centenaire de la Faculté de médecine vétérinaire of the Université de Montréal, and from Charles River Laboratories Montreal ULC.

REFERENCES

1 Ali BH, Bashir AK, Mugamer IT, Tanira MO: Gentamicin nephrotoxicity in the rat: influence of age and diabetes mellitus. Hum Exp Toxicol 1996:15(1):51-55.
2 Chandler MJ, Smith PJ, Samuelson DA, MacKay EO: Photoreceptor density of the domestic pig retina. Vet Ophthalmol 1999:2(3):179-184.
3 De Schaepdrijver L, Rouan MC, Raoof A, et al.: Real life juvenile toxicity case studies: the good, the bad and the ugly. Reprod Toxicol 2008:26(1):54-55.
4 Duarte DM: Use of juvenile animal studies to support oncology medicine development in children. Reprod Toxicol 2015:56:97-104.
5 EMA: Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications. London, UK: european Medicines Agency; 2008.
6 FDA: Guidance for Industry: Nonclinical Safety Evaluation of Pediatric Drug Products. Rockville: U.S. Food and Drug Administration,; 2006.
7 Hendrickson A, Hicks D: Distribution and density of medium- and short-wavelength selective cones in the domestic pig retina. Exp Eye Res 2002:74(4):435-444.
8 Herman EH, Zhang J, Chadwick DP, Ferrans VJ: Age dependence of the cardiac lesions induced by minoxidil in the rat. Toxicology 1996:110(1-3):71-83.
9 Kostic C, Arsenijevic Y: Animal modelling for inherited central vision loss. J Pathol 2016:238(2):300-310.
10 Middleton S: Porcine ophthalmology. Vet Clin North Am Food Anim Pract 2010:26(3):557-572.
11 Remick AK, Catlin NR, Quist EM, Steinbach TJ, Dixon D: Juvenile Toxicology: Relevance and Challenges for Toxicologists and Pathologists. Toxicol Pathol 2015:43(8):1166-1171.
12 Shively JN, Epling GP, Jensen R: Fine structure of the postnatal development of the canine retina. Am J Vet Res 1971:32(3):383-392.
13 Shrader SM, Greentree WF: Gottingen Minipigs in Ocular Research. Toxicol Pathol 2018:46(4):403-407.
14 Smelser GK, Ozanics V, Rayborn M, Sagun D: Retinal synaptogenesis in the primate. Invest Ophthalmol 1974:13(5):340-361.
15 Soellner L, Olejniczak K: The need for juvenile animal studies--a critical review. Regul Toxicol Pharmacol 2013:65(1):87-99.
16 Van Cruchten S, Vrolyk V, Perron Lepage MF, et al.: Pre- and Postnatal Development of the Eye: A Species Comparison. Birth Defects Res 2017:109(19):1540-1567.
17 Vézina M: Comparative ocular anatomy in commonly used laboratory animals. In: Weir A, Collins M, eds. Assessing Ocular Toxicology in Laboratory Animals: Humama Press; 2013: 12–17.
18 Vrolyk V, Desmarais MJ, Lambert D, Haruna J, Benoit-Biancamano MO: Neonatal and Juvenile Ocular Development in Gottingen Minipigs and Domestic Pigs: A Histomorphological and Immunohistochemical Study. Vet Pathol 2020:300985820954551.
19 Vrolyk V, Haruna J, Benoit-Biancamano MO: Neonatal and Juvenile Ocular Development in Sprague-Dawley Rats: A Histomorphological and Immunohistochemical Study. Vet Pathol 2018:55(2):310

Dosing and routes of administration in Göttingen Minipigs for toxicology studies

By Kirsten Rosenmay Jacobsen[1]

[1]Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark

Over the last 2 decades, a significant increase in the use of pigs and especially minipigs in translational research, including nonclinical toxicology and safety assessment, has led to an enhanced understanding of human diseases and improvement in human health. The advantages of using Göttingen Minipigs for translational research include a defined genome sequence and similarities to humans in terms of anatomy, physiology, and biochemistry. In addition, Göttingen Minipigs are significantly smaller than conventional pigs and other minipig breeds, has a gentle temperament, and high health status. For these reasons, they are widely used as a nonrodent species in many types of pharmacological and toxicological studies

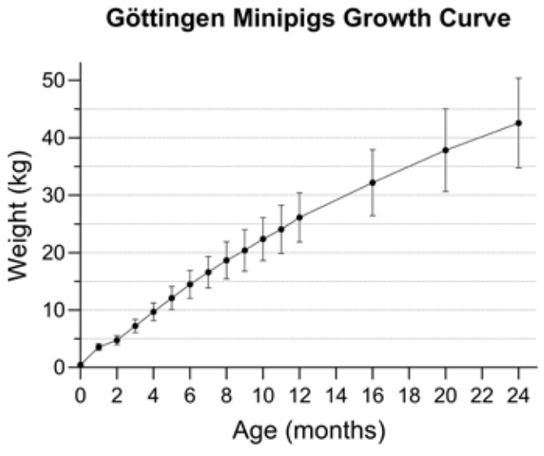
Handling

Prior to any study, Göttingen Minipigs should be acclimatized and socialized to humans and when relevant habituated or trained for the procedures. This reduces the level of stress inflicted on the animal, making it easier and safer to perform the study and reduces the risk of stress-induced bias.

It is common practice to use minipigs for toxicological studies at an age of approx. 4 months; an age where the animals weigh around 8-11 kg (graph 1). Minipigs this age can easily be picked up and carried on the arm for dosing. Restraining the minipig in a sling is also an option which is well tolerated by the animal. For some administration routes, fixation can be kept at a minimum and with little interference with the animal's behavior. Regardless of the route, the procedures should always be done with the optimal technique by well-trained staff and with a continuous focus on refinement.

Dermal

Dermal dosing is especially attractive in Göttingen Minipigs because of the similarities of the skin and corresponding tissue in humans. Microscopically, the epidermis is rather thick in both minipigs and humans, and with similar cellular turnover of 28-30 days. Also, there are many similarities in cellular composition e.g. identical number of cell layers in stratum corneum and in general similar immunological reactivity.



Graph 1
Weight curve for Göttingen Minipigs.

In terms of differences, the porcine skin is covered with apocrine glands that are located only in dedicated areas in humans (e.g. axilla and pubis area), and the minipig has a less vascularized dermis and a thicker subcutaneous fat layer. Furthermore, the porcine skin surface is slightly more neutral with a pH of 6-7 compared to the human skin with a pH closer to 5.

Macroscopically, the pale skin and the sparse and nonpigmented body hair make dermal effects easy to evaluate, and Göttingen Minipigs are, therefore, ideal for dermatotoxicology studies in most laboratories.

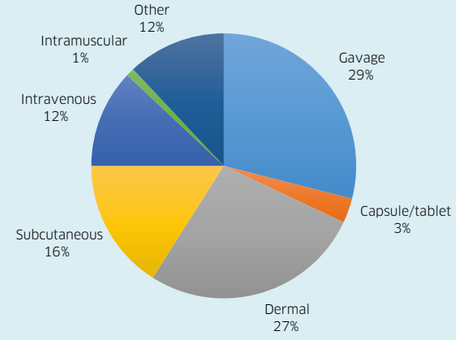
Dermal dosing is one area where minipigs can easily be trained to cooperate, and this greatly reduces stress levels for both the staff and the animal).

Subcutaneous

Subcutaneous dosing is commonly used, and the minipig offers major advantages for this route due to similarities with human subcutaneous tissue. In humans, a subcutaneous injection is often made by injecting into a skinfold, leading to injection into subcutaneous fat. In minipigs, it is also possible to inject into subcutaneous fat by injecting behind the base of the ears. Alternatively, once the minipig is around 6-8 months old, it is possible to make a skin fold similar to that of humans and inject into this. When creating a skin fold in other species, e.g. rodents, the injection will typically be into the loose connecting tissue and not the fat as in minipigs and humans. If desired, it is also possible to inject into such loose connective tissue in minipigs, for instance in the medial thigh region. The difference in subcutaneous tissue type can be important when assessing local toxicity, as such differences can lead to differences in compound distribution and tissue reaction toward the formulation.

Oral

The oral route is one of the most commonly used for dosing of minipigs. The minipig is a true omnivore as are humans and there are many anatomical and physiological similarities in the gastrointestinal system. Minipigs have similar gastric cell types, and the gastric pH in the fasted minipig is more comparable to



"At Charles River Copenhagen the usual route of administration in Göttingen Minipigs is either oral, dermal, subcutaneous or intravenous. However, we continue to work with our clients to support their needs and we continue to expand our capabilities and include new routes of administration, e.g. vesicular infusion and intraduodenal capsule delivery."

Jeanet Løgsted, General Manager,
Charles River Copenhagen.

Graph 2
Accummulated use of different routes of administration at Charles River Copenhagen.

that in humans as opposed to other species despite being more alkalic in general. Intestinal villi exhibit the same cell types as found in human small intestine as well as Peyer's patches and secretions are also like in humans. Furthermore, pH changes and transit time in the small intestine are similar to that of humans (about 4 hours). The large intestine is arranged in a series of coils and does not resemble the anatomy of humans, however the loops have no functional implication. It is important to be aware that the gastric emptying of the minipig is longer than the gastric emptying observed in humans, but this can be reduced by removing straw bedding (and potentially replacing it with another enrichment type) during the overnight fast.

To ensure proper dosing by gavage, a dosing chair can be helpful. Sometimes, orally administrated drugs or compounds can be given in the food, hidden in treats or capsules, or given directly from a syringe. However, this may not work for less palatable drugs or compounds and there are numerous study-related reasons why dosing must be controlled by oral gavage. Oral gavage is typically stressful for the minipig, but it can be reduced e.g. by dosing in a sling, or with correct fixation of the animal to allow a quick and smooth procedure. Regardless of the technique for oral dosing, the minipig is much less inclined to vomit as a response to various compounds and formulations compared to dogs.

Intravenous

The ear or saphenous vein are the most used sites for a single intravenous injection in the minipig. For multiple injections, a catheter is typically placed in the previously mentioned veins or more central veins with the catheter fixated externally or tunneled to a decided injection area/port. Consideration should be given to the properties of the compound and vehicle in deciding whether a peripheral vein is the right route of dosing or catheterization of a large vessel is more appropriate. Several types of catheters can be used for intravenous access in Göttingen Minipigs, depending on the specific study needs. An educational package describing some of the many options is available on the Ellegaard Göttingen Minipigs website (minipigs.dk).

A vascular access port can be placed under the skin or alternatively a vascular access button can be surgically implanted providing multiple catheters in the same animal. This provides the opportunity to dose and collect blood from different vessels and allows for long-term vascular access. With the vascular access button, no skin penetration is needed during dosing or blood sampling and the procedures can be done while the minipig is relaxed in a sling or with little fixation by a single person.

Miscellaneous

Because of the convenient size of Göttingen Minipigs all routes of administration and durations of study can be utilized. This applies, but is not limited, to intramuscular, intraperitoneal, intraocular, intravaginal, buccal, and inhalation dosing. Furthermore, ports or catheters into the desired organ can be surgically implanted allowing direct dosing at the target area e.g. the bladder.

For more details on dosing in toxicology and references, please see the paper "Göttingen Minipigs as large animal model in Toxicology" by Duelund & Mikkelsen, Biomarkers in Toxicology (Second Edition), 2019, Pages 75-89 (DOI: 10.1016/B978-0-12-814655-2.00003-7).

Norecopa: Prepare for better science

By Adrian Smith[1,2,3]

[1]Norecopa, Norway | [2]The Danish 3R Centre, Denmark | [3]The Danish Committee for the protection of animals used for scientific purposes, Denmark

Norecopa is Norway's consensus platform for replacement, reduction and refinement of animal experiments, founded in 2007. The name comes from the fact that Norecopa is a member of ecopa, an umbrella organisation which supports national platforms that have representatives in their governing bodies from all the 4 stakeholder groups: Regulators, research, industry and animal welfare.

Who is Norecopa?

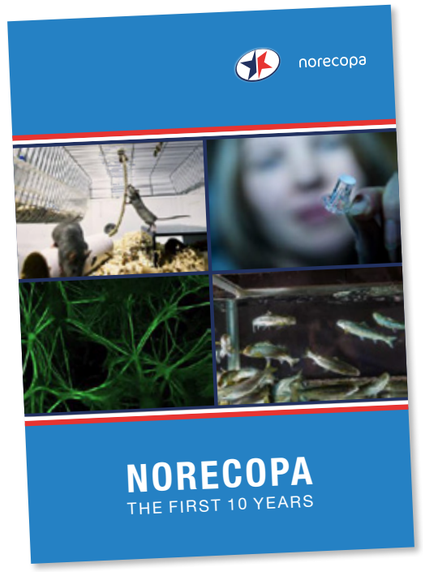
Norecopa is an independent member organisation representing four major stakeholders in animal research:

- Government and regulatory authorities
- Research and teaching
- Industry
- Animal protection and welfare

Norecopa functions as a centre of competence on questions concerning the 3Rs and attempts to achieve its goals through consensus between the stakeholders by functioning as a National Consensus Platform.

The primary aim of Norecopa is to promote the use of the 3R's, and hence contribute to increased knowledge of:

- *Replacement* of animal experiments by alternatives
- *Reduction* of the number of animals used in experiments
- *Refinement* of animal experiments to reduce suffering, increase animal welfare and increase the value of the experiments



Picture 1
Norecopa has accomplished a lot since the establishment in 2007. Download the publication from norecopa.no.

What Norecopa does

Norecopa aims to be a "one-stop-shop" for information about animal research and welfare. The Norecopa website consists of over 9,000 pages and currently gets over 300,000 hits each year - this figure has increased by 20-40% annually in recent years.

Databases
The website contains a number of databases for those planning animal research: these include one on guidelines for animal use, one on literature within lab animal science, and one with an overview of alternatives and supplements to the use of animals in teaching and training. All these databases have been seamlessly embedded in the website, together with an intelligent search engine, so only one search is necessary. Recently, a Refinement Wiki was added to the website. This is for the dissemination of advice and knowledge which often circulates on discussion forums but which does not get published in scientific papers. The wiki aims to fill this gap.

Species specific information
The website contains sections on specific groups of animal species, so that those who, for example, are mostly interested in farm animals can go directly to that section. All the relevant educational resources produced by Ellegaard Göttingen Minipigs of which Norecopa is aware, have been cited on the website.

International meetings
Norecopa arranges international consensus meetings about the care and use of animals in research, and has produced its own guidelines and positions statements on specific areas.

Webinars and online meetings
One of the positive side-effects of the covid-19 pandemic has been an upsurge in the number of webinars and virtual meetings about animal research and testing. Norecopa has for many years maintained a comprehensive Webinars & Meetings Calendar, updated several times a week. This calendar is now larger than ever before and also contains a page with links to recordings of presentations.

Newsletter
Norecopa issues an English-language newsletter 7-8 times a year, with information about the latest developments worldwide within research animal science and welfare. The newsletters are archived so that they are searchable by Norecopa's search engine. This section of the website also includes a newsfeed from European media about animal research and testing.

Göttingen Minipigs Magazine | 59 | 2020
Science

PREPARE for better science

Norecopa's motto is "PREPARE for better Science", whether that be with or without the use of animals. The word PREPARE refers to the PREPARE guidelines published by Norecopa in 2018. These guidelines provide a checklist for scientists planning experiments which may involve animal use, supported by comprehensive webpages with more information and references for each of the 15 main topics on the checklist.

PREPARE covers all stages from literature searches to collaboration with the animal facility and detailed advice on all stages of a project, including experimental design. The PREPARE paper was published under Open Access for free downloading, and has already been viewed or downloaded over 16,000 times. The PREPARE checklist has been translated into 22 languages so far and can be downloaded free of charge.

Norecopa has produced a 3-minute cartoon film to illustrate the importance of early planning and collaboration, using the aviation industry as an example. The film has optional subtitles in several languages:

Picture 2
Cartoon film by Norecopa illustrating the importance of planning and cooperation when doing research involving research animals. Links to the film are available from norecopa.no/PREPARE/film

USEFUL LINKS TO NORECOPA'S WEBSITE

The Norecopa website:
norecopa.no
Norecopa - The first 10 years:
norecopa.no/media/8091/the-first-10-years.pdf
The PREPARE guidelines:
norecopa.no/PREPARE
All translated versions of the PREPARE checklist:
norecopa.no/PREPARE/prepare-checklist
If the PREPARE checklist is not available in your language, contact adrian.smith@norecopa.no
Refinement Wiki:
wiki.norecopa.no
International consensus meetings:
norecopa.no/meetings
Webinars & Meetings Calendar:
norecopa.no/meetings/webinars-and-meetings-calendar
Species selection, farm animals:
norecopa.no/species/farm-animals
Information about minipigs:
norecopa.no/species/farm-animals/minipigs
Newsletter:
norecopa.no/news/newsletters
Research and testing in European media:
norecopa.no/news/newsfeed

Do you know of resources which should be mentioned on Norecopa's website? Contact adrian.smith@norecopa.no

The 3R principles explained

Replacement alternatives
Methods which permit a given purpose to be achieved without conducting procedures on animals.

Reduction alternatives
Methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures, or for obtaining more information from the same number of animals

Refinement alternatives
Methods which alleviate or minimise potential pain, suffering or distress, and which enhance animal well-being.

Table 1
Find more information on norecopa.no/alternatives/the-three-rs

Göttingen Minipigs Magazine | 59 | 2020
Science

PREPARE

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[c], Kristine E. Aa. Hansen[d] & Trond Brattelid[e]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].
PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

1. **Formulation of the study**
2. **Dialogue between scientists and the animal facility**
3. **Quality control of the components in the study**

The topics will not always be addressed in the order in which they are presented here, and some topics overlap. The PREPARE checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at **https://norecopa.no/PREPARE**.
The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, harm-benefit assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (replacement, reduction, refinement) and the 3Ss (good science, good sense, good sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a harm-benefit assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
6. Facility evaluation	☐ Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. ☐ Discuss staffing levels at times of extra risk.
7. Education and training	☐ Assess the current competence of staff members and the need for further education or training prior to the study.
8. Health risks, waste disposal and decontamination	☐ Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. ☐ Assess, and if necessary produce, specific guidance for all stages of the project. ☐ Discuss means for containment, decontamination, and disposal of all items in the study.
(C) Quality control of the components in the study	
9. Test substances and procedures	☐ Provide as much information as possible about test substances. ☐ Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	☐ Decide upon the characteristics of the animals that are essential for the study and for reporting. ☐ Avoid generation of surplus animals.
11. Quarantine and health monitoring	☐ Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	☐ Attend to the animals' specific instincts and needs, in collaboration with expert staff. ☐ Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	☐ Develop refined procedures for capture, immobilisation, marking, and release or rehoming. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, reuse or rehoming	☐ Consult relevant legislation and guidelines well in advance of the study. ☐ Define primary and emergency methods for humane killing. ☐ Assess the competence of those who may have to perform these tasks.
15. Necropsy	☐ Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

References

1. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Brattelid T. PREPARE:Guidelines for Planning Animal Research and Testing. *Laboratory Animals,* 2017, DOI: 10.1177/0023677217724823.
2. Kilkenny C, Browne WJ, Cuthill IC *et al.* Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PloS Biology,* 2010; DOI: 10.1371/journal.pbio.1000412.

Further information
https://norecopa.no/PREPARE / post@norecopa.no / @norecopa

Göttingen Minipigs Magazine | 59 | 2020

Working with Göttingen Minipigs

Why did you choose a career within biomedical research, specifically toxicology?
Biology always came naturally to me, all back to my high school years. I found both biology, chemistry, and science easy to understand, and I enjoyed studying these topics. So, it felt natural to use this as a steppingstone to build a career. However, becoming a toxicologist was more by chance as I did not have a preselected interest in toxicology specifically.

How and when were you introduced to minipigs as a large animal species?
Actually, I did not become aware of the minipig as a non-rodent alternative until the end 1990s. Back then, pigs were mainly considered as farm animals, and it was a long and gradual process of moving the pig away from that biased perception. In 2002 I moved to Denmark to start a new CRO job, and their specialty is with minipigs. Their way of working changed my perception of animal species selection, and as it turned out, this move changed everything for me moving forward.

Explain the value you believe Göttingen Minipigs bring to biomedical research.
As a scientist, you are obliged to do the best science possible. To make sure that the medicines you develop are safe and effective you must do appropriate experimentation and choose the correct animal species, which can deliver the results you are looking for. Therefore, it is important to have a wide range of species to select from. Göttingen Minipigs deliver options, as they are a well characterised animal model and due to their availability. Having the minipig available and having good reliable quality animals means that scientists can do research in the best possible way.

About Andrew Makin Preclinical Consulting Aps
Andrew has spent his entire career specialising in toxicology and pharmacology working in the CRO industry. Based on this expertise, he started his own consulting services in 2019 to advise companies with their preclinical projects.

Andrew Makin Preclinical Consulting specialises in preclinical and toxicology advisory and consultancy services to companies developing pharmaceuticals, medical devices, and other products in the stages prior to clinical trials or marketing. Though Andrew has experience with various rodent and non-rodent species, he today focusses on minipigs.

Visit andrewmakin.dk for more information.

In focus

Name Andrew Makin
Function Founder and CEO at Andrew Makin Preclinical Consulting Aps

Education
BA in Biology and Psychology, MSc in Applied Zoology.

Background
Andrew launched his career working with the CRO Huntingdon Life Sciences. Later, he moved to Denmark and was employed by Scantox (later Charles River Laboratories), after which he decided to start his own consulting services.

Engagements
Andrew recently celebrated his 40-year anniversary and has contributed with countless papers, articles, posters, and presentations to the scientific community. In 2019 he was appointed Göttingen Minipigs ambassador for his high-level knowledge dissemination and promotion of Göttingen Minipigs in biomedical research, and for his characterization, validation, and development of Göttingen Minipigs disease models.

What fascinates you the most about Göttingen Minipigs?
Göttingen Minipigs are unique because they are pathogen free, genetically well defined, and have a reliable background. Rat and mouse strains have been characterized over a number of years. Dogs have tended to evolve, but Göttingen Minipigs do not change much, which makes it possible to compare results over time.

You recently celebrated your 40-year anniversary in the industry. What do you believe has been the highlight(s) of your career so far?
Most of my career I have worked with CROs, and you rarely get to know what happens to the projects after you are done with them, which is not so motivating. Personally, the highlights of my career include becoming a Göttingen Minipigs Ambassador, which was a great recognition. Also, being invited to talk at conferences and seminars means that your peers choose you for something they believe you are good at. This kind of recognition which you receive from other scientists makes an impact.

How do you believe that you/your work makes a difference?
In any project it is important that the piece you are involved in functions and you always do the best you can. If you do something wrong or make any shortcuts, it can lead to someone making the wrong decision. You have to know where your own abilities stop and when to ask for advice. It has to be a team effort where you benefit from every individual's strong side. This makes a huge difference, and it is something I have always lived by and passed on to all the people who I have trained and co-worked with. Also, my work can ensure that the regulatory requirements are done properly and to the best of the researchers.

Looking into the future, how do you think animal research will develop within the next 25 years, and is it realistic to believe that we can do scientific research without the use of animals?
In some ways the industry is very innovative, but at the same time it is also very conservative, and progress happens very slowly. Animal research will still be done in a similar format in 25 years, even though a lot of work and effort is put into developing alternative methods and replacing the use of animals. I truly admire this work and what is trying to be achieved, but to be honest I do not see it happen any time soon. Animal studies started many years ago, and though techniques are different today they will still be in progress 25 years from now. That said, huge work is done in in vitro testing, which helps validate the animal testing that we do. This way you can see what the compound does before going into live animals and ensure that animal testing is done in the best possible way. So, it is right to believe that we can do scientific research without the use of animals in the future, but we must be realistic about the time frame and not delude ourselves that it will happen tomorrow.

Where have we seen the biggest development in regulatory guidelines from 40 years ago until today?
The biggest regulatory change has been the introduction of the ICH guidelines. Back when I started, we did studies that satisfied the American, European, Japanese guidelines respectively, meaning we performed numerous studies. ICH has been a huge breakthrough in that perspective. Since the inception in 1990 the number of animals needed for regulatory research has been reduced drastically. Another important development is the introduction of specific guidelines for pediatric medicines. These were not introduced until 20 or so years ago. Juvenile animal studies are important because they demand for special testing requirements. Typically, they are only performed in rodents. Minipigs also have a role to play in this, for example following the implementation of new food safety guidelines in the EU, where juvenile studies are required to make sure foods for infants are safe.

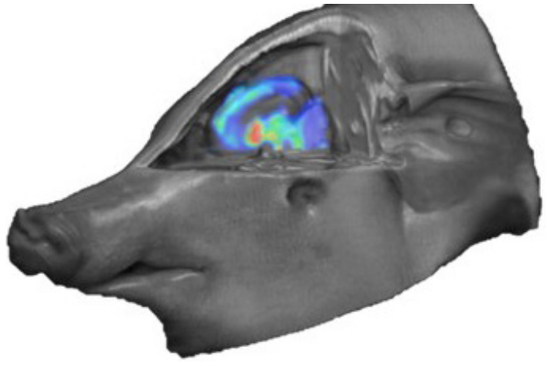
In vivo imaging of stem cell therapy in parkinsonian minipigs

ABOUT STUDY INSIGHTS: 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:
Anne M. Landau, Associate Prof. in Translational Neuroscience | Department of Nuclear Medicine and PET and Translational Neuropsychiatry Unit at Aarhus University, Denmark

What is the study about?
We use a non-invasive brain imaging method called positron emission tomography (PET), which provides in vivo measures of molecular targets. By combining PET neuroimaging with surgical, behavioural and post-mortem methods we are working towards validation of large animal models of neurodegenerative disease and the assessment of the efficacy of putative neuroprotective therapies over time in Göttingen Minipigs models.

What is the purpose of the study?
Parkinson's disease (PD) is a slowly, progressive neurodegenerative movement disorder for which there is no cure. The motor symptoms of PD, which include slowness and stiffness, are associated with a loss of the chemical messenger, or neurotransmitter, dopamine. Our overarching aim is to develop a chronic, slowly progressive large animal model of PD that recapitulates key pathological and symptomatic features of the human disease. Such a model would be valuable for the testing of therapies, and for the validation of novel imaging biomarkers of PD-relevant molecular targets, which can provide clues for early disease detection.



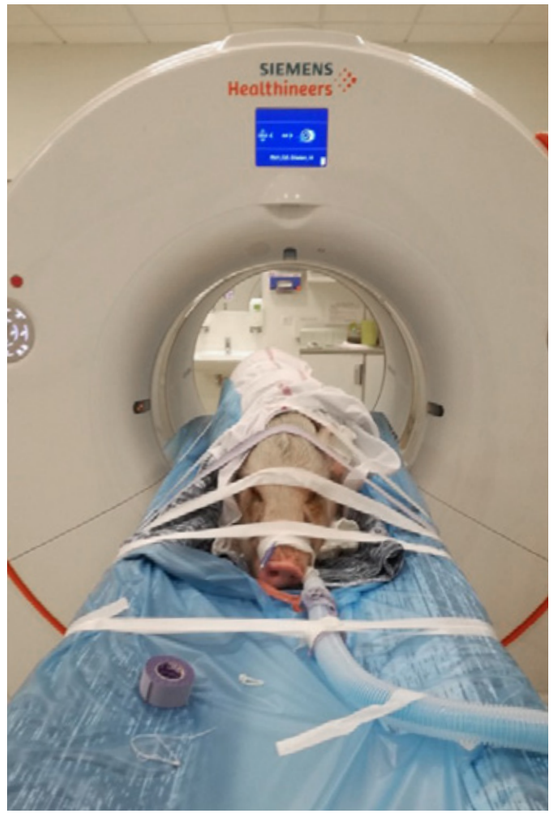
Picture 1
3D MRI reconstruction of the minipig head with PET brain activity superimposed.
Image provided by Postdoc Thea Pinholt Lillethorup.

We have induced the onset of PD mechanisms in Göttingen Minipigs using toxins, inhibition of the clearance system of the brain as well as by the overexpression of alpha-synuclein, a protein which aggregates in PD, in order to understand the different pathological mechanisms of PD and their pathological and imaging characteristics. It is our goal to develop a Göttingen Minipigs model that can be studied in the prodromal phase, prior to onset of symptoms, in order to have the greatest chance of screening therapies that can be applied early enough to modify the disease course.

We are currently trialing human embryonic stem cells which are engineered to form functional dopamine neurons as potential dopamine replacement therapy in PD. In collaboration with the Lund Center for Stem Cell Research, led by Professor Malin Parmar, and the Center for Experimental Neuroscience (CENSE) group at Aarhus University, led by Professor Jens Christian Sørensen, and thanks to funding from Parkinsonforeningen, Lundbeck Foundation and Innovation Fund Denmark, our group at the Aarhus PET Center has imaged parkinsonian minipigs implanted in the striatum with human embryonic stem cells. By following the changes in dopamine neurotransmission of the minipigs using in vivo PET biomarkers and behavioural analyses of motor function, we can evaluate the therapeutic efficacy of stem cells at early and late stages after implant.

Why is it important?
Cell replacement therapy is an exciting one-shot approach for treating neurodegenerative disorders. The rapid progress that has been made in engineering stem cells to viable dopamine neurons in recent years allows the use of stem cell transplants for repair of damaged dopamine circuits and may obviate the need for daily oral drugs to treat PD. It is important that these cells can be trialled for safety and efficacy in an appropriate translational large animal model and that effects can be investigated longitudinally. It is also relevant to learn which non-invasive imaging biomarkers of molecular targets may be translatable to human cases.

Göttingen Minipigs Magazine | 59 | 2020
Study Insight



Picture 2
Minipig in a Siemens PET scanner at the Department of Nuclear Medicine and PET, Aarhus University Hospital, in Skejby.
Photo provided by PhD student Simone Larsen Bærentzen

What makes this study particularly interesting?
The development of minipig models of disease and therapy allows us to confirm imaging data using post-mortem brain samples removed at critical timepoints, not often possible in human studies. We are then able to use post-mortem tissue with a number of other methods, such as immunohistochemistry and autoradiography. Taking this one step further, we and our collaborators have the capabilities of coupling PET and in vivo microdialysis (where a catheter is placed in brain tissue to directly sample brain fluid and monitor neurotransmitters) in order to understand the relationship between PET radioligand binding and neurotransmitter release in real-time. This work, made possible by the use of the minipig as a large animal model, gives us possibilities to investigate novel ideas which would not be possible in human participants.

Moreover, thanks to these minipig models and the ability to trial neuroprotective therapies, we are now in a position to assess the influence on newly developed in vivo PET biomarkers, for example, of synaptic density or mitochondrial function.

Which challenges have you met during the study?
In our initial study of the potential therapeutic effects of stem cells in a parkinsonian minipig model, we encountered two major challenges. First, the neurotoxin model that we had selected (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)) did not show the behavioural deficits that were previously reported. We also found spontaneous recovery of the markers of dopamine neurotransmission over time, which would confound the in vivo assessment of the stem cells.

Second, the immunosuppression protocol, necessary so that the immune system of the minipig does not attack the foreign human stem cells, that we first trialled prior to stem cell injection was insufficient, and we did not observe survival of a large number of cells. In our current study, we have selected the unilateral 6-hydroxydopamine (6-OHDA) minipig model of PD which does not recover spontaneously, and have raised the dose and closely monitored the levels of immunosuppressant by taking blood samples every 3-4 weeks, and are hopeful for a better outcome.

How do you recommend going about species selection?
In the current study, the minipig was selected to bridge the gap between rodent experiments and the planned trials in humans. The selection of the most appropriate animal model is driven by multiple considerations of time, species, size, and cost. For PET neuroimaging, the size of the brain is important since we aim to study small brain regions at sufficient resolution using human scanners. Göttingen Minipigs have a gyrated brain, like that of humans, of a size similar to primates, but with lower cost and handling restrictions.

Other factors must be considered when species are chosen for preclinical studies or development of new in vivo PET biomarkers for human use, as they can influence data analysis and interpretation. Different species may have specific physiological and metabolic characteristics such as different liver enzymes, receptor conformations, polymorphisms, etc., that may affect the interpretation of data and the translation of the results of animal studies to the clinical setting.

Finally, a major issue for longitudinal studies in Danish farm pigs is the rapid growth that precludes chronic studies. To circumvent this issue, Göttingen Minipigs, specifically developed for long-term research studies, have proven to be an excellent model for our own research where survival studies are done for up to 18 months.

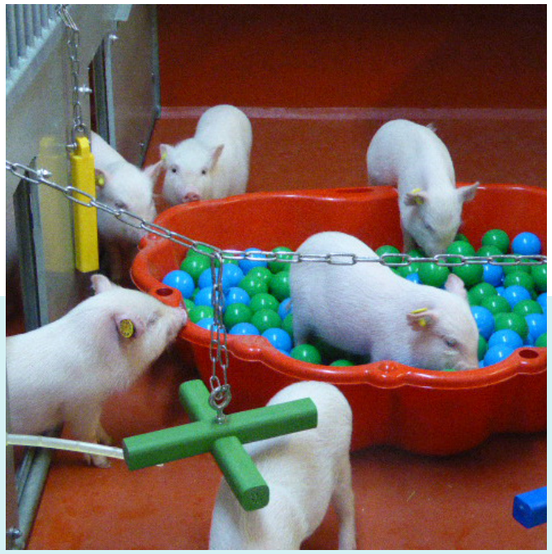
Spotlights

NC3Rs highlight on Göttingen Minipigs welfare

Four times a year the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) publishes their newsletter called Tech3Rs, featuring an interview with chosen animal technicians. The aim is to inspire how to implement the 3Rs effectively, by setting up a platform for animal technicians who work actively with the 3R principles at their individual establishments.

In the November 2020 edition, Animal Welfare Technician at Ellegaard Göttingen Minipigs A/S, Carina Christoffersen, shares the process of developing an enrichment room for Göttingen Minipigs. The challenge that initiated the project was, that the minipigs got bored with the toys they had available in their pens, even though they were exchanged on a daily basis. The solution was to establish an enrichment room, which can be compared to a playground for the minipigs, where they have a wide selection of enrichment at their disposal.

Read more in the newsletter on www.nc3rs.org.uk.



13 piglet litter

In average Göttingen Minipig sows carry litters of 7-8 piglets. But sometimes they surprise by delivering larger litters than usual.

This fall, one sow at Ellegaard Göttingen Minipigs delivered no less than 13 liveborn piglets in one litter. "A litter of this size is very rare, and even though this particular sow has a history of delivering large litters this is noticeable. We have only had 5 litters of this size in 2020, equalling 0,67% of all litters" explains Sofie Nørrelund Kirchhoff, Animal Caretaker at Ellegaard Göttingen Minipigs A/S.

All piglets were healthy at birth but to make sure none would be neglected and suffer from natural selection, some of the piglets were moved to other less fortunate sows. "It is common practice to relocate some of the piglets to make sure they all receive the maternal care that they need. Usually, the sows foster 7-8 piglets depending on their age, condition and own litter size" Sofie Nørrelund Kirchhoff elaborates.

The largest litter registered at Ellegaard Göttingen Minipigs in 2020 was of 14 piglets.

Göttingen Minipigs Magazine | 59 | 2020

Minipigs as a well-characterized animal model for NASH

Göttingen Minipigs can be, and has already been, used to support NASH research by providing well-characterized, diet-induced Göttingen Minipigs with severe non-alcoholic steatohepatitis (NASH).

At Ellegaard Göttingen Minipigs they have experience with this approach and now offer these models on a contract basis. To follow the development of disease progression in each individual NASH minipig, multiple blood samples as well as liver biopsies can be provided at specified timepoints.

Read more in the article "NASH-inducing Diets in Göttingen Minipigs" published in the May/June edition of the Journal of Clinical and Experimental Hepatology on sciencedirect.com. For more information about diet induced models in Göttingen Minipigs like NASH, obesity and atherosclerosis contact Peter Vestbjerg, Head of Business Development at Ellegaard Göttingen Minipigs A/S, at pve@minipigs.dk.

Studying to become an Animal Caretaker

Becoming an Animal Caretaker of research animals is an education that takes dedication and a love for animals and their welfare.

Production Manager at Ellegaard Göttingen Minipigs A/S, Søren Vangsgaard, employs and mentors 5 Animal Caretaker trainees. "If you expect to keep hiring Animal Caretakers in the future, you are obliged to contribute to the continuous education of new staff, whether they end up working at your own facility or decide to move on. We take this social responsibility very seriously and are proud to be part of the education and training of each student who works with us", he explains.

One of the Animal Caretaker trainees, Sidse Lærke Madsen, has been with Ellegaard Göttingen Minipigs for 2,5 years and will graduate in the summer 2021. She appreciates the experience of being part of the day-to-day business and says: "I have become much more confident in my profession during my traineeship. Being part of a team and being appointed my own areas of responsibility has made me grow both personally and professionally. Also, I have come to know what everyone else in the company does, what their functions are, and general knowledge of the research animal business."

Apart from taking proactive part in caring for the minipigs trainees are offered to participate at Ellegaard Göttingen Minipigs' research facility. Sidse Lærke Madsen explains: "Being involved in the activities in the research barrier means that I will know how to prepare the minipigs for surgeries, know the procedures that follow, etc. These are great skills to learn and help make me attractive to future employees."

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.*

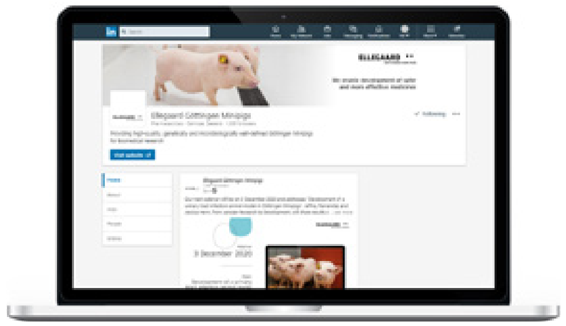
Subscribe to news and invitations

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

Follow on LinkedIn

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

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



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
Hypersensitivity reactions to nano-biopharmaceuticals: Foundation of a minipig model	12 January 2021 10 am CET**	Janos Szebeni | SeroScience Ltd., Hungary	bit.ly/EGMweb210112
Anticancer drug development comparison of toxicity in minipig and mouse	3 February 2021 10 am CET**	Sally-Anne Reynolds | Sequani Limited, United Kingdom	bit.ly/EGMweb210203
Drug metabolism in Göttingen Minipigs: Critical information for species selection in drug safety testing	24 February 2021 10 am CET**	Steven Van Cruchten | University of Antwerp, Belgium	bit.ly/EGMweb210224
Imaging techniques for Göttingen Minipigs with PET, MRI and CT	16 March 2021 10 am CET**	Aage Kristian Olsen Alstrup | Aarhus University Hospital, Denmark	bit.ly/EGMweb210316

* *Central European Summer Time*
** *Central European Time*

Implantation of Rat Vascular Access Buttons in GÖTTINGEN MINIPIGS

Adrian Zeltner, Ellegaard Göttingen Minipigs, Denmark

To download the poster as pdf please scan this code:

Introduction:

Infusion and serial blood sampling are often important technical aspects of an experimental design. Superficial vessels in the minipig are few and frequently accessing them is a challenge. Although minipigs have a convenient size for handling, restraint and venepuncture can be stressful and affect blood parameters. Therefore, when experiments require infusion or frequent blood sampling, catheterization is often the best option, both ethically and scientifically.
The implantation of Vascular Access ports and Seldinger Catheters is described and published in various variations. To add another option when choosing the optimal study design, we tested the Rat Vascular Access Button™ in Göttingen Minipigs. The button allows up to three catheters to be connected and opens the possibility to sample and dose via one device but trough different catheters. This has, to our knowledge, not been done before.

Minipig in pen after surgery

Button implanted behind the ear

Taking a blood sample

Blood sampling performed by one person with Minipig in a sling

Placement of catheters, colours explained in text below

Rat Vascular Access Button ready to implant. (above)

VAB after 2 months at necropsy. Dracon cuff is completely ingrown in the surrounding tissue. (right)

Material and Methods:

Three studies were conducted:

1. Pilot
Proof of concept, determine best approach and type of catheter.
4 male Göttingen Minipigs (14 -15 kg). Rat Vascular Access Buttons (Instech Laboratories, Inc. USA) with three ports for three catheters. Each Minipig had three catheters implanted from a midline incision:
1. In the left carotid artery; two Minipigs in cranial direction and in two in caudal direction. A 3fr PU catheter, with a bead at 3 cm was inserted to that length and fixed with a purse string suture and tissue glue. (red)
2. In the left internal Jugular vein. This vessel runs alongside the carotid artery. It was ligated and a 3fr PU catheter, with two lateral perfusion holes, at 3 cm and 2 mm apart was inserted to a length of 7-8 cm, so the tip rested in the vena cava. (blue)
3. In the right internal jugular vein. A 3fr PU catheter, was inserted in the same manner as in the other vein. (yellow)

2. Housing
Feasibility test of Group housing
4 male Göttingen Minipigs (17 -20 kg). Rat Vascular Access Buttons (Instech Laboratories, Inc. USA) with one port, no catheter implanted.
Some caps were camouflaged with a less obvious colour and some had a bitter tasting additive. It seemed to make no difference. If caps fell of it was mainly due to lack of magnetic adhesion or intensive movement.

3. Main study
Increased animal number applying results from pilot. (ongoing)
16 Göttingen Minipigs (10 -12 kg). 8 males , 8 females. 8 with Rat Vascular Access Buttons of one port/catheter, 8 with Buttons of two ports/catheter.
The left external Jugular vein was dissected and after ligation one ore two 3fr PU catheters were 7 cm inserted. If there was two catheter the insertion was trough the same puncture and insertion length was 7 and 9 cm.
Catheters were secured around the vessel with a modified Miller knot, followed by square knots. Another tie cranial of the bead was placed an the catheter was further secured with the ends of the initial ligature.
Incision closed in three layers using PDS II, continuous pattern, the last intradermal. (green)

All catheters were tunnelled to a subcutaneous pocket created behind the left ear and then connected to the button. The incision of the implantation site was closed in three layers, anaesthesia discontinued and the minipigs left to recover. After one week of post op care, including antibiotics and analgesia, vascular access was tested. In the group housing study the animals were euthanized after that period. For a period of two or three months the buttons were accessed roughly every 7 days to test patency and functionality. At the end of that period the animals were sacrificed, and a necropsy of the affected area performed.

Results:

The incisions in the neck and at the site of the button healed really well and no signs of infection or other complications were observed. The felt collar of the button was grown in the subcutaneous tissue to seal the exit site completely.
All ports worked fine, but some typical catheter related issues appeared after some time. The table below shows clearly that the standard catheter in the vein worked best.

Catheter issues withdraw		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Catheter 1 Artery	Cranial A		S	R	B	B	B	B	B
	Cranial B			S	R	R	R	R	R
	Caudal C			R	R	R	R	R	R
	Caudal D		R	R	R	R	R		
Catheter 2 Perfusion holes	A		S	B	B	B	B	S/B	B
	B	S	S	S	R	B	S	S	R
	C		R	B	B	R	R	B	B
	D		R	R		R	B		S
Catheter 3 Standard	A						R	R	
	B								
	C								R
	D					S			

Results of 8 weeks testing. S– slow withdraw; R– fine after rinsing; B–blocked even after rinsing

In the group housing study no complications were noted. Behavior of the pigs showed that group housing can be an option, however some of the caps fell off, leaving the ports unprotected. For the main study the caps were modified with two magnets, but that was still not satisfactory. Further modification by adding a lock screw helped, however not in all cases. The pigs do not interfere with the button itself but unprotected ports are not ideal. Technically a screw cap would solve the problem but they are not available from the supplier.

In the main study the incisions in the neck and at the site of the button healed well and no signs of infection were initially observed.
Four weeks post surgery we found some infections around the button in 6 cases, 3 of them were minor and disappeared quickly after treating with antibiotics. One remained an bit longer and two animals were euthanized (5 and 6 weeks after surgery) as the button was no longer embedded thoroughly in the tissue.
Necropsy showed that the infection was limited around the button with a folded up Dracon cuff. It might have been due to improper implantation in the fat rather than below the fat layer. The tip of the catheter was where it was supposed to be, in the vena cava and no signs of clots or thrombi were observed.
In some cases function of both catheter stopped at the same time.

Artery Perfusion Standard

Rinsed Catheter tips at end of the pilot study. All catheter with perfusion holes had large fibrin deposit, whereas standard ones were clean. Artery catheters had some fibrin attached.

Conclusion and Discussion:

It can be concluded that Rat Vascular Access Buttons™ can successfully be implanted in Minipigs and provide long term vascular access. The buttons can accommodate 1-3 catheters which gives the opportunity to infuse and sample trough the same unit without cross-contamination. Implantation is a permanent procedure; catheters can be explanted but this is not possible for the VAB as it is ingrown.
Infection around the VAB can possibly be reduced by making sure the Dracon cuff rests between the muscle and the fat layer and the skin is snug around the neck of the VAB.
Accessing is painless and if the Minipigs are trained to be in a sling, only one person is required to perform procedures.
There are typical catheter related issues, that can be minimized by proper catheter handling, namely locking under positive pressure.
Preliminary results of the main study show, that the assumed advantage of having two catheters (having a backup if one is blocked) is not necessary true. Blocked catheters can become patent again after some time or repositioning the animal.

All procedures were carried out in compliance with current laboratory guidelines for good conduct and the project was conducted with approval of the Danish Animal Experiments Inspectorate according to Danish law. Animals were housed at approved facilities and taken care of by qualified persons.

ELLEGAARD GÖTTINGEN MINIPIGS

The 14th Minipig Research Forum

Facility of Ellegaard Göttingen Minipigs A/S

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.

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.

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

Göttingen Minipigs Magazine | 59 | 2020

A preview into 2021

One year ago, we were looking forward to a prosperous New Year 2020. At Ellegaard Göttingen Minipigs we had just ended a successful 2019, a year of celebrating 50-years with Göttingen Minipigs and was amazed by the support and interest we received during our global roadshows and launch of scientific webinars. Now we look forward to yet another new year.

New event facility under construction.

Though 2020 turned out a lot different than expected, opportunities, initiatives, and new ways of conducting business also presented itself. Successful 2020 Initiatives from Ellegaard Göttingen Minipigs included the launch of the Göttingen Minipigs Magazine and the continuation of our very popular webinar series - which has come to stay.

Looking into 2021, we are excited to announce the launch of more new initiatives. These include:

- The introduction of the Ellegaard Göttingen Minipigs Academy (more information below)
- New event and seminar facilities at our location in Dalmose, Denmark
- A brand new website

...and much more.

Practical training sessions in our fully equipped surgical suite.

NEW IN 2021:

Ellegaard Göttingen Minipigs Academy

The Ellegaard Göttingen Minipigs Academy will facilitate seminars and workshops targeted for employees working within laboratory animal science on various topics concerning Göttingen Minipigs. In addition to the scheduled courses, on-demand lectures and hands-on training tailored to your specific needs and interests is available. Training will be conducted at the premises of Ellegaard Göttingen Minipigs A/S in the well-equipped seminar room, or in our animal and laboratory facilities. Training can also be conducted at the participants' own location.

The overall purpose of the Ellegaard Göttingen Minipigs Academy is to increase the knowledge of Göttingen Minipigs. It provides a platform for continuous education of relevant competences and development of new skills ultimately improving animal care, welfare, and use of Göttingen Minipigs. The seminars will contain both theoretical and practical training and will be conducted by experts within their respective fields, all with years of experience with Göttingen Minipigs.

Look out for the planned seminars:

Seminar 1	Vascular Access
Seminar 2	Animal Training
Seminar 3	Anaesthesia and analgesia
Seminar 4	Göttingen Minipig behavior, welfare, and veterinary care

If you wish to be notified when the dates are available, subscribe to news at minipigs.dk/sign-up-for-news.

Göttingen Minipigs Magazine | 59 | 2020

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 ellegaard@minipigs.dk.

Mahan B, Antonelli MA, Burckel P, et al.
Longitudinal biometal accumulation and Ca isotope composition of the Göttingen minipig brain
Metallomics. 2020 Oct 21;12(10):1585-1598
Doi: 10.1039/d0mt00134a
https://pubmed.ncbi.nlm.nih.gov/33084720/

Allmendinger A, Fischer S
Tissue Resistance during Large-Volume Injections in Subcutaneous Tissue of Minipigs
Pharm Res. 2020 Sep 4;37(10):184
Doi: 10.1007/s11095-020-02906-9
https://pubmed.ncbi.nlm.nih.gov/32888065/

Fu Y, Xu J, Tang Z, et al.
A gene prioritization method based on a swine multi-omics knowledgebase and a deep learning model
Commun Biol. 2020 Sep 10;3(1):502
Doi: 10.1038/s42003-020-01233-4
https://pubmed.ncbi.nlm.nih.gov/32913254/

Doyle-Eisele M, Brower J, Aiello K, et al.
Developing and Comparing Models of Hematopoietic-Acute Radiation Syndrome in Göttingen and Sinclair Minipigs
Int J Radiat Biol. 2020 Oct 19;1-15
Doi: 10.1080/09553002.2020.1820604. [Epub ahead of print]
https://pubmed.ncbi.nlm.nih.gov/32909874/

Zaer H, Glud AN, Schneider BM, et al.
Radionecrosis and cellular changes in small volume stereotactic brain radiosurgery in a porcine model
Sci Rep. 2020 Oct 1;10(1):16223
Doi: 10.1038/s41598-020-72876-w
https://pubmed.ncbi.nlm.nih.gov/33004849/

Vrolyk V, Desmarais MJ, Lambert D, Haruna J, Benoit-Biancamano MO
Neonatal and Juvenile Ocular Development in Göttingen Minipigs and Domestic Pigs: A Histomorphological and Immunohistochemical Study
Vet Pathol. 2020 Nov;57(6):889-914
Doi: 10.1177/0300985820954551.
https://pubmed.ncbi.nlm.nih.gov/33021158/

Paolone G
From the Gut to the Brain and Back: Therapeutic Approaches for the Treatment of Network Dysfunction in Parkinson's Disease
Front Neurol. 2020 Oct 7;11:557928
Doi: 10.3389/fneur.2020.557928
https://pubmed.ncbi.nlm.nih.gov/33117258/

Poirier Y, Becker S, Decesaris C, et al.
The Impact of Radiation Energy on Dose Homogeneity and Organ Dose in the Göttingen Minipig Total-Body Irradiation Model
Radiat Res. 2020 Nov 10;194(5):544-556
Doi: 10.1667/RADE-20-00135.1
https://pubmed.ncbi.nlm.nih.gov/33045066/

Hulse EJ, Smith SH, Wallace WA, et al.
Development of a histopathology scoring system for the pulmonary complications of organophosphorus insecticide poisoning in a pig model
PLoS One. 2020 Oct 14;15(10):e0240563
Doi: 10.1371/journal.pone.0240563
https://pubmed.ncbi.nlm.nih.gov/33052985/

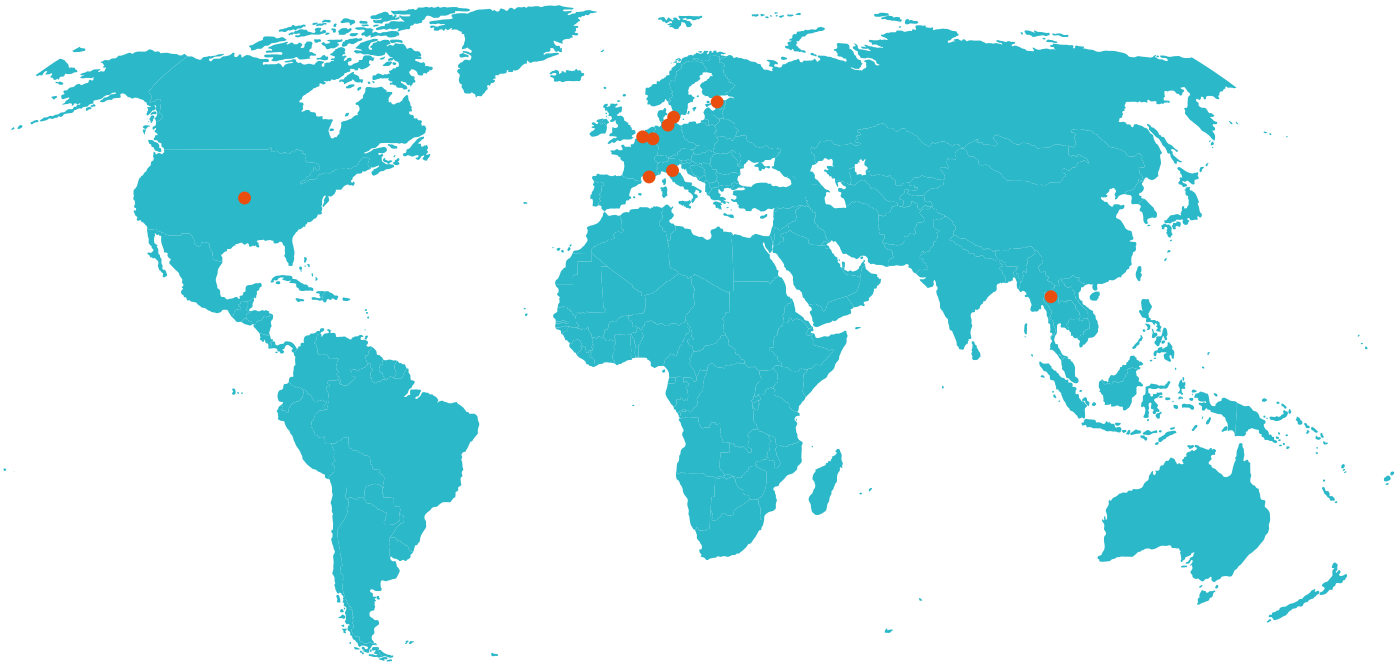
Song W, Sun W, Chen L, Yuan Z
In vivo Biocompatibility and Bioactivity of Calcium Silicate-Based Bioceramics in Endodontics
Front Bioeng Biotechnol. 2020 Oct 29;8:580954
Doi: 10.3389/fbioe.2020.580954
https://pubmed.ncbi.nlm.nih.gov/33195142/

Curtasu MV, Tafintseva V, Bendiks ZA, et al.
Obesity-Related Metabolome and Gut Microbiota Profiles of Juvenile Göttingen Minipigs—Long-Term Intake of Fructose and Resistant Starch
Metabolites. 2020 Nov 12;10(11):456
Doi: 10.3390/metabo10110456
https://pubmed.ncbi.nlm.nih.gov/33198236/

Zaer H, Fan W, Orlowski D, et al.
A Perspective of International Collaboration Through Web-Based Telecommunication–Inspired by COVID-19 Crisis
Front. Hum. Neurosci. 2020 Nov 23
Doi: 10.3389/fnhum.2020.577465
https://www.frontiersin.org/articles/10.3389/fnhum.2020.577465/full

Xu Y, Curtasu MV, Bendiks Z, et al.
Effects of dietary fibre and protein content on intestinal fibre degradation, short-chain fatty acid and microbiota composition in a high-fat fructose-rich diet induced obese Göttingen Minipig model
Food Funct. 2020 Nov 24
Doi: 10.1039/d0fo02252g. [Epub ahead of print]
https://pubmed.ncbi.nlm.nih.gov/33231591/

Where to meet us in 2021



CONGRESS / CONFERENCE	DATE	LOCATION
IAT	9-25 March	*Online event*
SOT and ToxExpo	14-18 March	*Online event*
Scand LAS	13-15 April	Tallinn, Estonia
Janssen Juvenile Toxicology Symposium	22-23 April	Beerse, Belgium
AFSTAL	26-28 May	Marseille, France
Tierschutz-Tagung Travemünde	8-9 June	Lübeck, Germany
ESLAV ECLAM	21-24 June	Bologna, Italy
AFLAS	21-27 June	Chiang-Mai, Thailand
World Congress (WC11)	22-26 August	Maastricht, Holland
EUROTOX	26-29 September	Copenhagen, Denmark
Minipig Research Forum (MRF)	29 September - 1 October	Dalmose, Denmark
AALAS	16-21 October	Kansas City, Missouri, USA
STP-I	TBA	TBA
SPS	TBA	TBA
GV-SOLAS	TBA	TBA
3R's Research and Progress	TBA	TBA
LASACON	TBA	TBA
CALAS	TBA	TBA

Europe and Asia
Ellegaard Göttingen Minipigs A/S
Sorø Landevej 302,
DK-4261 Dalmose,
Denmark
Tel.: +45 5818 5818
ellegaard@minipigs.dk

North America
Marshall BioResources
North Rose, NY 14516, USA
Tel.: +1 315 587 2295
Fax: +1 315 587 2109
infous@marshallbio.com

Japan & Taiwan
Oriental Yeast Co. Ltd.
3-6-10, Azusawa, Itabashi-ku
Tokyo, 174-8505, Japan
Tel.: +81 3 3968 1192
Fax: +81 3 3968 4863
fbi@oyc.co.jp

Korea
WOOJUNGBIO
B-3F, 145 Gwanggyo-ro,
Yeongtong-gu, Suwon, Korea
Tel.: +82 31 888 9369
Fax: +82 31 888 9368
ljhong@woojungbio.kr