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Göttingen Minipigs
MAGAZINE

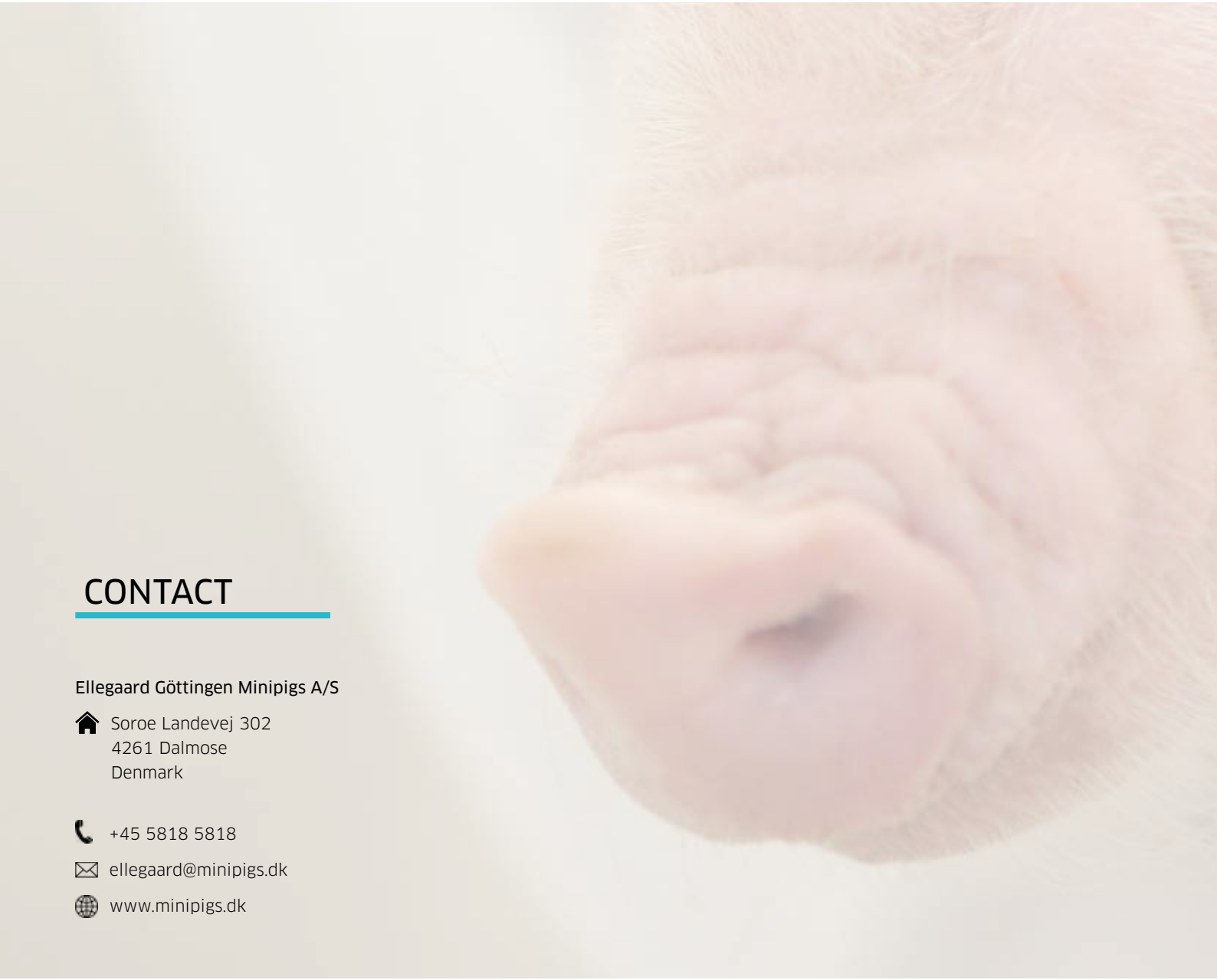
ELLEGAARD
GÖTTINGEN MINIPIGS

Dear reader

As we close the books for 2022, it comes naturally to reflect on the year that has passed. And what a year it has been.

After years with conference cancellations due to Covid-19, we have finally been able to meet many of you in person at events around the world during 2022. It has been a pleasure discussing both current activities and plans for the future.

2022 also became the year, when Ellegaard Göttingen Minipigs hosted the Minipig Research Forum at our premises and welcomed over 100 participants committed to sharing their knowledge and experience in the use of minipigs in biomedical



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research. On behalf of Ellegaard Göttingen Minipigs, I am proud and honoured of the engagement and positive interest in our facility. Thank you.

In September, we were proud to present the new humanised Göttingen Minipigs with the publication of "A humanized minipig model for the toxicological testing of therapeutic recombinant antibodies" in Nature Biomedical Engineering. The genetically altered Göttingen Minipigs model is yet another step with which we pursue our goal of enabling the development of safer and more effective medicines and treatments, and we are excited about new collaborations and projects based on this large animal model in the years to come.

Now, looking into 2023, we have more new initiatives coming. For now, I can unveil the introduction of the Göttingen Minipigs Academy, which is introduced on page 25, and not least the celebration of our 30-year anniversary, which will be marked throughout the year.

The team at Ellegaard Göttingen Minipigs and I look forward to many new projects, collaborations, and networking opportunities in 2023.

I wish you all a Happy New Year.

Martin Windfeld Velin, CEO
Ellegaard Göttingen Minipigs A/S

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Introducing a new line of Göttingen Minipigs

Göttingen Minipigs are particularly known for their small size, genetics and health status, and are selected for all aspects of pharmaceutical research due to their similarity to humans and access to background data. Now a new line of Göttingen Minipigs has become commercially available.

10 June 2022 the red carpet was laid out to celebrate the introduction of the new Humanized IgG Göttingen Minipigs carrying a mini-repertoire of human genes and showing tolerance to a broad range of human antibodies. "This is a breakthrough for the possibilities of testing therapeutic antibodies in large animal models, as we now have an important alternative to non-human primates. Göttingen Minipigs are widely accepted as a pre-clinical non-rodent model and have proven its suitability both in terms of handling and dosing and data validity. Also, it is easy to incorporate the 3Rs when working with Göttingen Minipigs, as they are adaptable, easy to train, and you can get many data points from one animal", says Andres Eskjær Jensen, Study Director at Ellegaard Göttingen Minipigs A/S.

Following the introduction of the Humanized IgG Göttingen Minipigs, a round table meeting was conducted in Copenhagen in November 2022 and another is scheduled in Basel in February 2023. The meetings address future perspectives for the new Göttingen Minipigs line. Evaluating on the introductory events and the launch of the new strain of Göttingen Minipigs, Martin Windfeld Velin, CEO at Ellegaard Göttingen Minipigs A/S, says: "Introducing this new line of Göttingen Minipigs is a huge step,

Humanized IgG Göttingen Minipigs now commercially available

At Ellegaard Göttingen Minipigs' research facility it is possible to test your compounds and perform exploratory and preliminary non-GLP studies in Humanized IgG Göttingen Minipigs.

Download more information from bit.ly/about-HumGM or contact orders@minipigs.dk to discuss your opportunities.

Humanized IgG Göttingen Minipigs are bred at the fully AAALAC accredited barrier facility at Ellegaard Göttingen Minipigs in Denmark, and thus have a high health status comparable to standard Göttingen Minipigs. They are available in large uniform groups, sexually mature and ready to use.


ELLEGAARD

Humanized IgG Göttingen Minipigs
Now commercially available

Webinar on demand

Humanized Göttingen Minipigs for the toxicological testing of therapeutic antibodies

The safety and efficacy of most novel biologicals is evaluated in animal models. However, most human therapeutic antibodies trigger xenogeneic responses in wild-type animals and thus rapid clearance of the drugs, which makes in vivo testing of human antibodies challenging. Here we report the generation of Göttingen Minipigs carrying a mini-repertoire of human genes for the immunoglobulin heavy chains ϒ1 and ϒ4 and the immunoglobulin light chain κ. In line with observations in human patients, the genetically modified minipigs tolerated the clinically non-immunogenic IgG1κ-isotype monoclonal antibodies daratumumab and bevacizumab, and elicited antibodies against the checkpoint inhibitor atezolizumab and the engineered interleukin cergutuzumab amunaleukin. The humanized minipigs can facilitate the safety and efficacy testing of therapeutic antibodies.

and we are equally proud and excited about the scientific potential this presents for the future. The Humanized IgG Göttingen Minipigs represent an important step in our ambition to continuously enable development of safer and more effective medicines."

The Humanized IgG Göttingen Minipigs possess the same qualities as the standard Göttingen Minipigs, but has additional benefits due to the genetic modification:

Benefits of all Göttingen Minipigs:

- Small size
- Genetics
- Background data
- Similarity to humans
- Barrier bred in AAALAC accredited facility and with high health status
- Availability in large uniform groups

Additional benefits of Humanized IgG Göttingen Minipigs:

- Safety assessments of human IgG-based therapeutic antibodies (tAbs)
- Reflects the difference in immunogenicity of different approved tAbs
- Confirmed trait inheritance
- Immunocompetent

Open access to publication

The development of the new Humanized IgG Göttingen Minipigs has been fully described in the paper "A humanized minipig model for the toxicological testing of therapeutic recombinant antibodies", published in Nature Biomedical Engineering on 22 September 2022.
doi.org/10.1038/s41551-022-00921-2

Whitepaper

Humanized IgG Göttingen Minipigs for pre-clinical safety assessment of therapeutic antibodies

Genetically altered Göttingen Minipigs carrying a mini repertoire of human Ig-γ1/γ4 heavy and the human κ light chain genes show tolerance to a broad range of human antibodies. This provides a novel model for safety testing and an important and viable alternative for toxicology studies of therapeutic antibodies in non-human primates.

Download the whitepaper: bit.ly/whitepaper-HumGM

Behind the paper:

Humanised Göttingen Minipigs – an alternative animal model to non-human primates in the preclinical safety assessment of therapeutic antibodies

By Tatiana Flisikowska[1]

[1]Researcher at the Department of Molecular Life Sciences, Chair of Livestock Biotechnology, School of Life Sciences Weihenstephan, Technical University München, Freising, Germany.

Recent years have seen tremendous advances in the treatment of human disease, largely due to the development of biopharmaceutical drugs. Among these, the largest class are monoclonal antibodies (mAbs), which are currently used in therapies for chronic and severe diseases (e.g. cancer, inflammatory diseases). One of the primary concerns during the development of novel biopharmaceuticals is efficacy and safety.

Introduction

Selecting a pharmacologically relevant animal model for toxicological testing of human antibodies can be challenging. Although most of the safety testing is performed in rodents, the value of these models is limited for many reasons. For instance, anatomical and life span differences compared to humans make the data obtained in rodents difficult to translate to humans in terms of routes of application, pharmacokinetics and long-term toxicological assessment (1). Immunogenicity is generally assessed during animal trials within the context of an intact immune system. However, any human protein will likely cause an immune response in a test animal because of species differences. This can be circumvented by using transgenic animals that express the human protein and therefore recognise it as self. Any immune response raised will therefore be to the altered state of the recombinant protein. This, however, requires creating a separate transgenic animal line to evaluate each therapeutic protein. Such a strategy conflicts with the '3Rs' principles, whose aim, in addition to replacing (**R**eplace) and refining (**R**efine) is to limit the number of animals (**R**educe). Non-human primates (NHPs), for obvious genetic, anatomical and physiological similarities, are recognised as the most human-relevant animal species. However, their use in safety testing raises ethical and practical concerns. The EU legislation (2) imposes a legal obligation to reduce the number or even replace NHPs in research. The availability of other large animal systems for the assessment of the immunogenic and immunotoxic character of human therapeutic antibodies (Abs) would be a very valuable tool to enhance the pre-clinical safety predictability of this rapidly growing class of drug. The pig has many advantages for preclinical studies, being similar to humans in size, in the anatomy of many organ systems, and its physiological and pathophysiological responses. Pigs have a relatively short gestation time, large litter size, rapid maturation and ease of housing in pathogen-free conditions. For these reasons, the pig has emerged as a human-relevant animal species for translational research. Moreover, the immunological similarities to humans make the pig a prime candidate as a large animal immunologically tolerant to human Abs suitable for generating preclinical data translatable to the human system.

Humanised Göttingen Minipigs – The Beginning of Tomorrow

The story behind the generation of humanised Göttingen Minipigs dates back to 2015 when Dr Antonio Iglesias, a scientist working at Roche Pharmaceutical Research and Early Development, established a transgenic mouse line carrying a human IgG1 mini-repertoire (3). In contrast to most Ab-humanised mouse models, these transgenic mice elicit normal antibody responses to unrelated antigens while being immunologically tolerant to a large number of human IgG1 Abs. However, as mentioned above, many factors have limited their use for toxicology experiments. The Göttingen Minipig as the most common minipig strain is recognised by many pharmaceutical companies, as an alternative non-rodent model. Thus, Antonio contacted us with a project to create human-relevant Göttingen Minipigs expressing human immunoglobulin. The transgenic repertoire was generated using constructs previously expressed in transgenic mice, except that the IgH chain construct has been modified to now express both the IgG1 and the IgG4 isotypes, instead of the IgG1 isotype alone. Human IgG1 and hIgG4 were chosen as these are the two Ig isotypes most commonly used in human therapeutic mAbs (4). The second construct was modified to contain IgK gene segments only. Upon proper rearrangement, these gene elements should generate a repertoire of human soluble IgG proteins without interfering with the process of rearrangement and repertoire formation of endogenous porcine Ig proteins as this requires the expression of the membrane-bound human Ig, which is not included in the transgenic construct.

Production of genetically engineered founder Göttingen Minipigs

Founder animals were produced by somatic cell nuclear transfer (SCNT) in collaboration with Prof. Eckhard Wolf (Ludwig-Maximilians-Universität, LMU), who established this technology in pigs. The procedure for producing transgenic Göttingen Minipigs is presented in Fig. 1. Briefly, kidney fibroblasts isolated from a male Göttingen Minipig (provided by Ellegard Göttingen Minipigs company) were co-transfected with the IgH and IgK expression

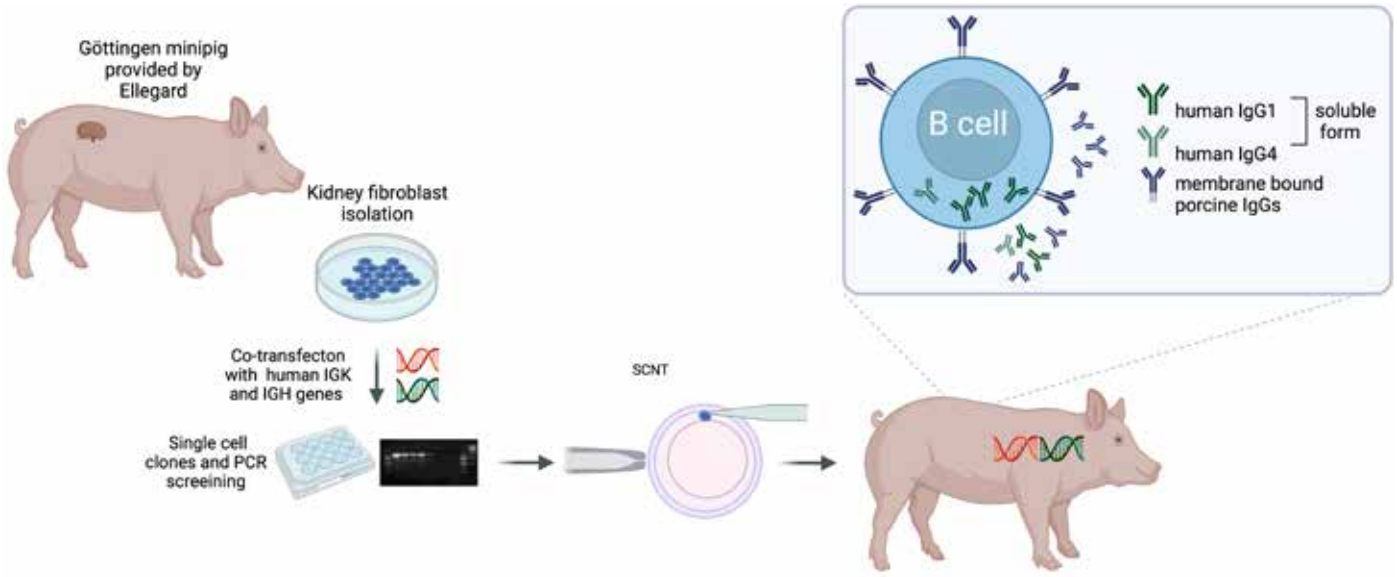

Figure 1
Generation of humanised Göttingen Minipigs. Illustration adapted from Flisikowska et al., 2022 (9). Created with BioRender.com

vectors together with a selectable marker. Single-cell clones were isolated and screened by PCR. Positive cell clones, which carried all three transgenes were pooled and used for SCNT. Eight male Göttingen piglets were born from two pregnancies.

Do transgenic animals maintain a normal immune response?

The animals were healthy and did not suffer any abnormally increased infection load, indicating that the normal immune response of the animals was not affected by the expression of human IGH and IGK transgenes. To confirm these findings in an experimental setting, some of the humanised minipigs were injected with T-cell dependent model antigen keyhole limpet haemocyanin (KLH) (4), the response to which is well-documented for Göttingen Minipigs (5). All treated minipigs (transgenic and wild-type) mounted an IgM and IgG response one week after immunisation, confirming that the transgene expression does not alter T-cell-dependent Ab responses to this protein. In a subsequent boosting experiment, the first dose of KLH was followed by rechallenging the animals with a second dose at day 35. The quick increase in KLH-specific porcine IgG titres after the booster immunization demonstrates that the humanized minipigs can also mount a memory response. Taken together this data showed that the expression of human IgG does not compromise the immune capacity of Göttingen Minipigs.

Are humanised Göttingen Minipigs tolerant to human antibodies?

To show the tolerance status of the humanised minipigs, we used bevacizumab and daratumumab, which are known to elicit low clinical immunogenicity in patients (6). As expected, in contrast to the wild-type, none of the transgenic animals showed an immune response after repeated application of bevacizumab and daratumumab.

Could transgenic minipigs predict the immunogenicity of human antibodies?

To answer this question transgenic and wild-type pigs were injected with atezolizumab or cergutuzumab, which are known to induce anti-drug antibodies (ADA) responses in 39% and 70% of patients, respectively (7,8). All minipigs developed ADA responses. The ADA titre was significantly lower for the humanized minipigs after atezolizumab treatment, reflecting the difference in immunogenicity between these two therapeutic Abs. The results obtained from the human IgG transgenic minipigs recapitulate those observed in humans and data from studies in hIgG1 transgenic mice (3,9).

For more details regarding the results and methods, please refer to the paper by Flisikowska et al. (2022) (9).

Conclusions

We could show that the humanised Göttingen Minipigs respond to immunogenic compounds and tolerate non-immunogenic Abs, providing an ideal model for testing safety and predicting possible side effects of therapeutic Abs.

The International Council for Harmonization (ICH) require conducting preclinical safety studies in relevant animal models– one rodent and one non-rodent species. The human IgG minipigs together with previously generated transgenic mouse lines meet these conditions. The newly derived humanized minipigs may help reduce or even replace NHPs in safety and efficacy testing.

Beyond the humanised Göttingen Minipigs

Translating advances in basic research into the clinic requires animal disease models, whose physiology and pathophenotype resemble that of humans as closely as possible, and so can provide reliable, representative and -most importantly- predictive information in preclinical trials. Genetically engineered mouse models for human diseases (e.g. cancer) are an invaluable source of knowledge but are not always the most suitable means of translating new findings into clinical applications. The shortcomings of the exclusive use of rodents in preclinical studies are now widely recognised. The regulatory agencies around the world require preclinical trial data from

Image 1
Pen with Humanized IgG Göttingen Minipigs.

non-rodent species. Thus, our group has a strong interest in providing the research community with novel large animal models for cancer research and has generated minipigs for a number of different cancer types (see https://www.btn.wzw.tum.de/research-interests.html, for existing oncopigs). Our oncopig models have already been used for preclinical studies to refine technologies for the detection of premalignant gastrointestinal lesions with biodegradable fluorescent nanoparticles (10) and a protease-activated near-infrared fluorescent probe ("smart probe) (11). Extending novel imaging methods to diseases lacking early diagnosis options, for example, pancreatic cancer, is the next logical step. As the pigs are relatively long-lived, important clinical parameters such as disease progression and remission, response or failure of therapy can be followed. If human therapeutic antibodies should be assessed in the porcine cancer model, a cross with the human IgG pig line would allow for testing the toxicity and also clinical efficacy.

We are further engaged in a national consortium entitled „Biology of xenogeneic cell, tissue and organ transplantation - from bench to bedside", for which we have produced several multi-transgenic and multi-knockout lines. These pig models provide a humanised glycosylation pattern and express human complement regulatory and anti-inflammatory transgenes. Our most-advanced lines are the 5x transgenic pigs that show the highest expression levels of the human complement regulatory genes CD46, CD55 and CD59, and expression of the human anti-inflammatory and anti-apoptotic genes (HO1 and A20). Further, they carry inactivation of the most-important xeno-reactive antigens, encoded by the genes GGTA1, CMAH and B4GALNT2, and SLAI, the porcine MHC class I. We currently provide these animals for numerous perfusion experiments and preclinical transplantation settings in non-human primates.

CONTACT INFORMATION

If you are interested in more information or discussing potential collaborations, please visit our website https://www.btn.wzw.tum.de/home.html or contact tatiana.flisikowska@tum.de.

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A small, convenient "on demand" Göttingen Minipig obesity model

By Jeanet Løgsted[1], Marianne Kronborg Bracken[1], Henrik Duelund Pedersen[2#], and Berit Østergaard Christoffersen[3].*

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Obesity is one of the most frequent non-communicable diseases. The prevalence has tripled since 1975 and in 2016 more than 650 million people worldwide were living with obesity (1). Obesity is associated with development of the metabolic syndrome, characterized by dyslipidemia with elevated triglycerides, decreased high-density lipoprotein cholesterol (HDL-C) in addition to insulin resistance and hypertension, which ultimately may develop into type 2 diabetes and cardiovascular disease (2). Hence, there is a huge need to develop new and efficacious anti-obesity medications, which creates a need for relevant and readily available preclinical obesity models.

The pig is a commonly used non-rodent model in metabolic research due to its many physiological and anatomical similarities with humans (3). The Göttingen Minipig has additional benefits due to its small size and widespread use in cardiometabolic research, in addition to the propensity of female and castrated male minipigs to develop obesity (4, 5, 6). However, typically the induction of a human-like degree of obesity, e.g. a body fat percentage of up to 40-50%, can take from 6-12 months depending on the sex of the pigs in addition to the diet composition/feeding regimen (7, 8), which is not very flexible in a drug development setting. Furthermore, in many of the previously described obesity studies in Göttingen Minipigs the animals have reached a body weight (BW) of around 80-100 kg (7, 8), which may impact animal welfare as it puts a lot of strain on the limbs and may result in lameness due to the overload. It also presents additional challenges for staff in the handling of the obese minipigs and for the requirements of housing conditions.

Groups (n=6)

1.	OVX Females	chow diet
2.	Castrated Males	chow diet
3.	OVX Females	HFD diet
4.	Castrated Males	HFD diet

The aim of the current study was to develop and characterize a small, convenient "on demand" Göttingen Minipig obesity model for testing new pharmaceutical anti-obesity treatments and for studying the physiological and metabolic changes occurring with obesity. The primary goal was to induce obesity with a human-relevant body fat percentage of at least 40-50% within 12 weeks. Secondary goals were to have insulin resistance and dyslipidemia, as well as a more manageable size of the pigs.

Animals. Twelve male and twelve female Göttingen Minipigs approximately nine weeks of age were castrated or ovariectomized (OVX) 11 days prior to study start. It has previously been described that both OVX female Göttingen Minipigs (7, 8) and castrated male Göttingen Minipigs (6) develop severe obesity when fed either a high energy diet or a chow diet ad libitum. In addition, OVX reduces the variation in food intake associated with the oestrous cycle, and hence, it was decided to include both OVX females and castrated males in the current study (Figure 1).

Figure 1
Study and group overview.
DEXA: dual-energy X-ray absorptiometry
HFD: High fat diet
OVX: ovariectomised
IVGTT: Intravenous glucose tolerance test
MMT: Mixed meal test. Days on x-axis.

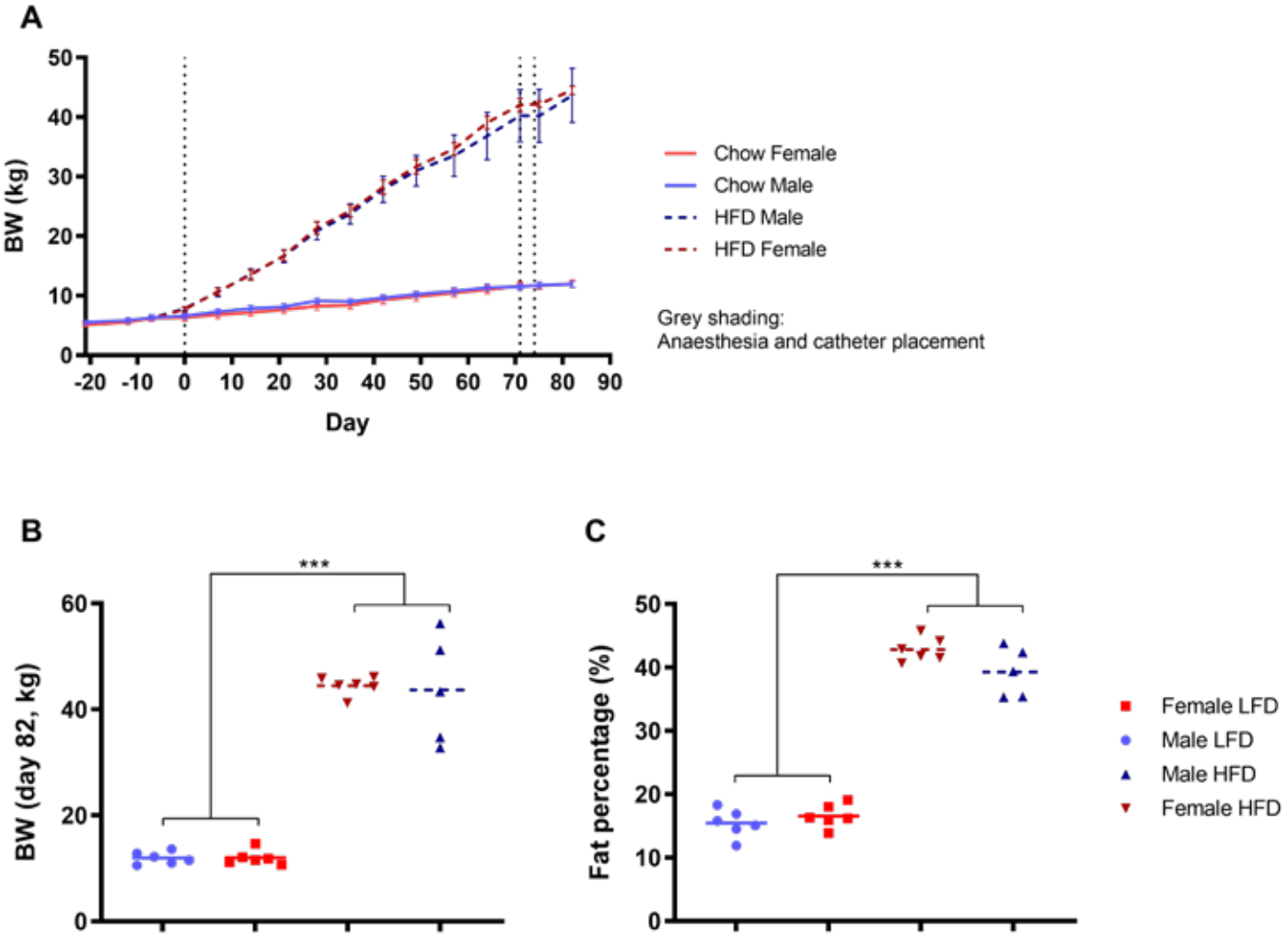

Figure 2
Body weight development (A), end study body weight (B) and end study body composition (C) in ovariectomised female and castrated male Göttingen Minipigs fed either chow diet or high fat diet (HFD). BW: body weight. Data are mean±SEM for (A) and mean and individual values (B and C). *** p<0.001.

Diet. The control animals were fed restrictedly twice daily with chow (SDS minipig expanded, Special Diets Services, Scanbur, DK) according to the breeder's recommendation, whereas the obese animals were fed with a custom-made high energy diet (Foulum, Aarhus University, Aarhus, Denmark) containing 25% fat, 0.5% cholesterol and 23% fructose for a total of 12 weeks. The obese animals were fed close to ad libitum for the first 8 weeks and switched to full ad libitum feeding for the last 4 weeks of the study (Figure 1).

Fasting blood samples were obtained at the end of the 12 weeks diet period for measurement of various metabolic parameters, such as plasma glucose, insulin, triglycerides, total cholesterol, LDL-C, HDL-C. Body composition was determined by dual-energy

Parameter [unit]	Females chow	Males chow	Females HFD	Males HFD	Significance#
Glucose [mM]	4.1±0.22	4.3±0.38	4.6±0.24	4.6±0.28	Ns
Insulin [pM]	28±8	21±	65±9	42±8	Diet***
HOMA-IR	0.84±0.27	0.65±0.17	2.09±0.31	1.46±0.29	Diet***
TG [mM]	0.72±0.15	0.50±0.07	0.66±0.09	0.56±0.12	Ns
Total cholesterol [mM]	1.89±0.09	1.78±0.15	3.03±0.51	3.50±0.47	Diet***
HDL cholesterol [mM]	1.33±0.05	1.11±0.09	1.70±0.36	1.98±0.34	Diet*
Non-HDL cholesterol [mM]	0.83±0.09	0.90±0.09	1.65±0.24	1.96±0.26	Diet***

Table 1
Fasting plasma parameters in ovariectomised female and castrated male Göttingen Minipigs fed either chow diet or high fat diet (HFD). Data are mean±SD. * p<0.05, ** p< 0.01, *** p<0.001. Ns=non-significant. #Two-way ANOVA. HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

X-ray absorptiometry (DEXA) scanning (Hologic Explorer, Santax Medico, Aarhus, Denmark) on the day of termination. In addition, a mixed meal test and an intravenous glucose tolerance test was performed at study end (data not shown).

The body weight development and the body composition are shown in Figure 2, whereas the fasting plasma parameters are shown in Table 1.

Overall results indicated no significant differences in any of the parameters between the castrated males and the OVX females on the respective diets. The HFD fed animals weighed approx. 2.5 times more than their lean counterparts at study end and had a body fat percentage of around 40% (Figure 2). However, the actual BW of the obese minipig was only 40-50 kg meaning half of the traditional obese models. As expected, there was no effect of diet feeding on fasting glucose, whereas HFD lead to increased fasting insulin and increased insulin resistance (evaluated by Homeostatic Model Assessment for Insulin Resistance, HOMA-IR) (Table 1). There were no differences in TG levels in the fasted state, but total cholesterol, non-HDL-C and to a lesser extent HDL cholesterol were all statistically significantly increased in the HFD fed animals.

Treatment with a long-acting glucagon like peptide 1 (GLP-1) receptor agonist twice weekly for 6 weeks in OVX female Göttingen Minipigs induced with obesity as described above and weighing approx. 30 kg at treatment start, gave rise to a significantly smaller weight gain compared to vehicle treated pigs ($p<0.001$) (Figure 3).

In summary, it was possible to induce severe obesity within 12 weeks of HFD feeding in OVX and castrated Göttingen Minipigs. The obese minipigs showed some degree of dyslipidemia and impairment in their glucose metabolism. Treatment with a long-acting GLP-1 analogue gave rise to a significantly smaller weight gain compared to vehicle, indicating the potential of the model for testing pharmacological anti-obesity treatments. Pros and cons of the model are indicated in Table 2.

Pros	Cons
Rapid induction of severe obesity (body fat~40%)	Neutering required to induce obesity/reduce variation in food intake
Small model – less compound and diet required compared to 80-100 kg pigs - easier to handle	Custom-made high fat diet required
Insulin resistance and hypercholesterolemia	Anti-obesity interventions will show reduced weight gain - not weight-loss
A positive control compound (a long-acting GLP-1 receptor agonist) results in a significantly lower BW gain	

Table 2
Pros and cons of the small, rapidly induced Göttingen Minipig obesity model.

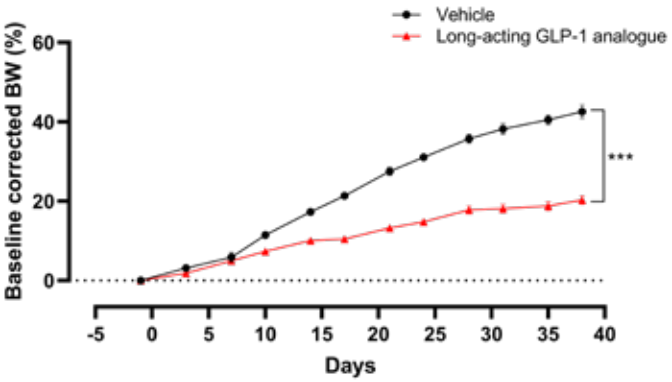

Figure 3
Six weeks of treatment with a long-acting GLP-1 receptor agonist given subcutaneous twice weekly in ovariectomised high fat diet-fed female Göttingen Minipigs gave rise to a significantly lower body weight gain compared to vehicle (n=6-12). Data are mean±SEM. *** $p<0.001$.

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Automated blood sampling in minipigs

By Malene Schrøder[1] and Sten Velschow[1]

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It is often a requirement during animal trials to do repeated blood sampling to measure changes either in the concentration of biomarkers or of a dosed compound in the blood. A general goal of animal studies is to minimize the number of animals used while providing high quality data. A general limitation in animal studies is the number of blood samples that can be collected from one given animal contra the number of timepoints the scientist would like.

Introduction

Repeated blood sampling is required in many research settings, e.g. during PK/PD studies and safety pharmacology studies. For some specific study designs it is necessary to have satellite groups of animals for blood sampling as this procedure affects negatively other sensitive end-points, like telemetry - or behavioral data.

Currently, manual procedures for serial sampling in larger animals (dogs, pigs and NHP) requires lifting the animal into a sling, training the animal to sit on a table, other restraint procedures or eventually implanting long-term intravenous catheters.

Serial manual blood sampling includes the following common challenges:

- It is labor-intensive, time consuming and may introduce human errors. It requires involvement of numerous staff to both fixate the animal and manually handle each blood sample.
- Complex logistics are involved when handling wet blood samples in terms of labelling, centrifugation, and freezer facilities.
- Data quality is affected by many possible sources of error during collection, handling, and storage of samples.
- Reproducibility of data is challenging, as the exact test set-up is dependent on diligent time schedules and success during each sampling occasion.
- Occupational health is a factor when handling large animals, working round the clock and being under time pressure.
- Animal welfare and ethics dictate how many samples you can collect from one animal in a trial.

Automated serial blood sampling devices provide an alternative to manual serial blood sampling. There are different products on the market which are specifically aimed for smaller laboratory animals like rodents. Culex® (Bioanalytical Systems, Inc.) and ABS2 (Instech Laboratories, Inc.) are examples of automated samplers which share the common feature that they withdraw blood from a tethered, freely-moving animal. These systems are not directly usable for larger animals.

For larger laboratory animals, i.e., rabbits, NHPs, dogs and minipigs, Fluispotter®, a wearable automated blood sampling device which allows for up to 20 hours of unattended serial sampling of maximum 20 blood samples, can be used.

Implementation and use

Equipment

Fluispotter is a small device which requires minimal investment in infrastructure in your animal facility. In Figure 1. you see Fluispotter, which consists of:

A Control System, which is a reusable, rechargeable, programmable wearable equipment
B Software for programming of Control System and generation of data log for GLP records
C Cartridge, which is a sterile consumable for single use. The Cartridge has a 6 ml reservoir for flushing solution, and it collects blood samples as dried blood spots
D Catheter, which is a sterile consumable for single use; 45 cm long, multi-lumen for recirculation and limited blood loss

When operational, Fluispotter weighs 75 grams and is mounted on the back of the animal.

Figure 1
From left: Fluispotter Catheter with markings, attached to Fluispotter Cartridge. Fluispotter Control Unit and to far the right the assembled Fluispotter. In front, paper-reel with dried blood spot samples.

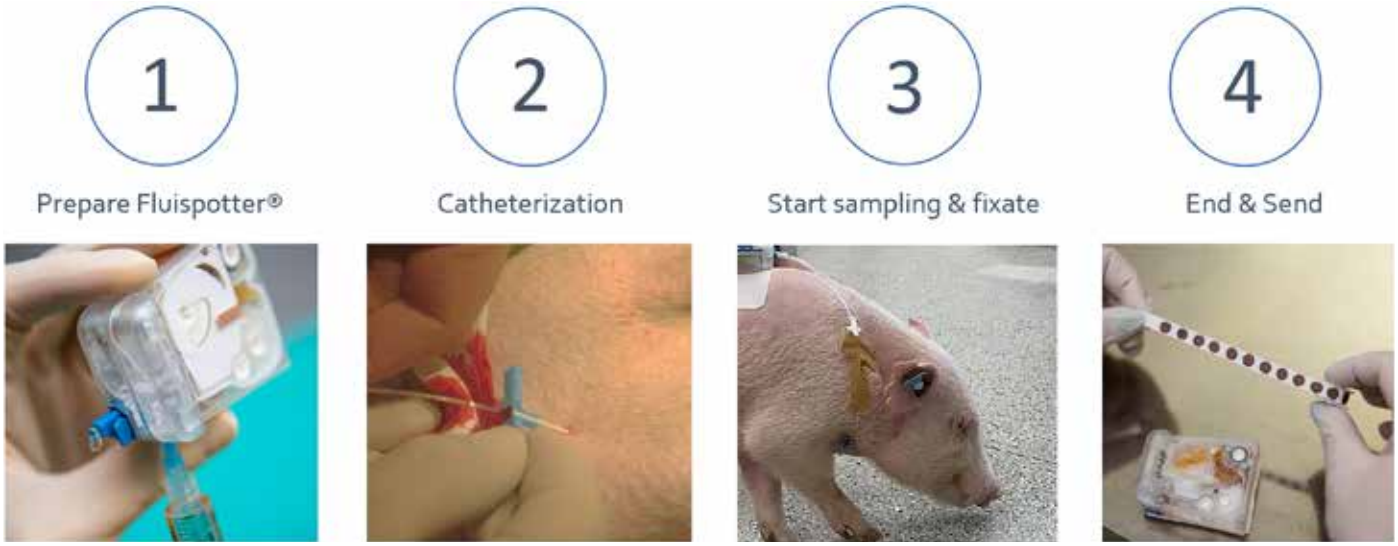


Figure 2
Fluispotter procedure in 4 easy steps.

Methods
The whole sampling procedure is completed in four overall steps (Figure 2).

1. Preparation of Fluispotter

The sampling schedule is created in Fluiconnect™ software, which must be installed on a PC. Via Fluiconnect the Fluispotter Control System is programmed and charged.

Fluispotter Cartridge™ and Fluispotter Catheter™ must be preloaded with flushing solution before use; 4% Sodium Citrate solution is recommended.

Fluispotter Control is clicked together with Fluispotter Cartridge and the system is ready for use.

2. Catheterization

Catheter placement is performed using sterile principles while the minipig is placed under full anaesthesia in a supine position for a short surgical procedure.

Fluispotter Catheter must be placed in a large enough vein, preferably the jugular vein. A guide catheter is introduced into the jugular vein via the Seldinger technique:

- a guidewire is introduced into the jugular vein via an 18 Ga needle
- a small skin incision close to the guide wire is made using a scalpel
- a guide catheter is placed over the wire
- the guide wire is removed, and the guide catheter flushed with sterile salt water
- Fluispotter Catheter is placed through the guide catheter into its desired depth, which is dependent on the size of the minipig
- the catheter is connected to the Fluispotter Cartridge
- the guide catheter is then carefully removed from the vein while ensuring the Fluispotter Catheter stays in place

An alternative is to place a 16 Ga Peel-Away Introducer directly in the jugular vein, placing the Fluispotter Catheter and removing the peel-away.

To ensure that the Fluispotter Catheter is not misplaced during the sampling period, it should be fixed to the skin. This can be done either by suturing a catheter clamp to the skin or alternatively with a strong plaster like Fixumull®.

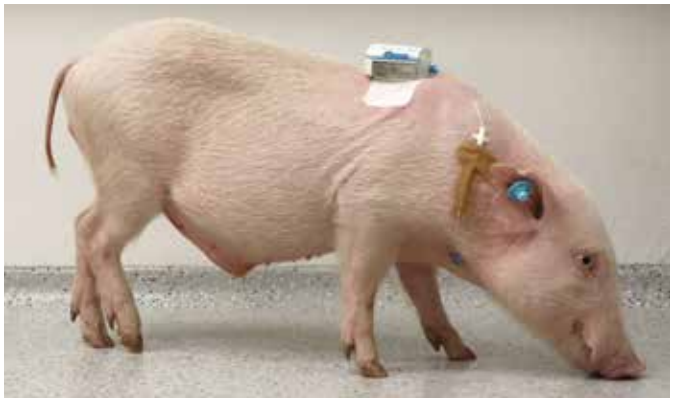
3. Start sampling and fixate Fluispotter

Fluispotter is started by pushing the button. Once the catheter is fixed to the skin and Fluispotter is mounted on the back (see Picture A) it is recommended to bandage the neck and chest using a flexible self-adherent bandage.

4. End & Send

Once Fluispotter has completed the desired sampling profile, the animal must be restrained shortly (see Picture B) to remove the catheter from the vein and detach the Fluispotter and the adhesive patch from the back of the pig.

The cartridge contains a paper strip with the dried blood samples. The paper strip is easily removed from the cartridge



Picture A
Göttingen Minipig wearing Fluispotter.

and can be stored in a dry place at room temperature until analysis is performed in the lab. The cartridge and the catheter should be disposed as medical waste.

The Control Unit requires no maintenance, but regular recharging of the battery.

Output

Data log

When reconnecting Fluispotter Control Unit to the PC, it will be possible to obtain a data log showing exactly when the blood samples were collected and the exact volume of each sample.

Dried blood spots

Fluispotter collects up to 20 volumetric dried blood spots (DBS) of 3-10 µL over the course of up to 20 hours. The accuracy of the blood sampling volume is ± 0.3 µL (1).

Due to the design of the catheter tip and the constant recirculation of blood, carry-over is < 5% when sampling at 10 min. intervals. Method validation experiments must be performed to establish the validity of the analytical assay for the compound you want to measure in the blood sample.

Discussion

Dried blood spots

DBS was introduced in the early 1960's in neo-natal screening for metabolic disorders. The main feature for the use of DBS was the low blood volume requirement, but the technique has proven valuable as a very easy way of blood sampling and a very robust way of storing blood samples.

Since the early 2000's the development of LC-MS/MS equipment and immunoassays provided very sensitive assays, that has initiated an intense development of DBS based assays as the ability to generate analytical results from small blood samples could improve the ethical use of laboratory animals, as it allowed less discomfort and limited the number of animals used.

The use of DBS did experience a set-back in drug development as FDA, in the draft Guidance for Industry Bioanalytical Method Validation of 2013, stated that exposure data based on DBS should be supported with correlative studies based on traditional sampling (2). The statement was repeated in the final version of 2018 (3). However, because of the advantages of DBS compared to wet samples, the use and implementation is still ongoing, mainly in research and clinical applications and in the area for home testing purposes. Today, numerous biomarkers, small molecules, large molecules, and antibodies can be recovered from DBS.

The ICH Guidance E14/S7B for the non-clinical evaluation of QT/QTc Interval and Prolongation and Proarrhythmic Potential (4)

The recently published final ICH Guidance document considers it best practice to preferable use the same animal species to obtain telemetry data and complementary information on systemic exposure levels (toxicokinetic) for in vivo QT studies. The Guidance document used the principles of 3R as an argument for this recommendation.

Due to the limited availability of dogs and NHP and the general understanding that minipigs are a good model for humans regarding heart monitoring, minipigs are being more widely used in telemetry studies.

As Fluispotter is wearable and automated animal trials can be performed without having to worry about human presence stressors or constraint of the animals during sampling thereby affecting the outcome of the trial. A study in Beagle dogs (5) shows cortisol levels significantly lower than the normal reference interval, indicating that Fluispotter® opens new ways of reducing pain, fear, and discomfort for animals in animal trials, and gives the research community a new powerful tool to further 3R efforts.

Fluispotter is the best available solution for larger laboratory animals to refinement and reduction, better animal welfare and improved quality of data.

Picture B
Gentle handling of Göttingen Minipig during removal of Fluispotter.

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3 Guidance for Industry Bioanalytical Method Validation Final (May 2018).
4 E14 and S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential - Questions and Answers. ICH Guidance (Aug 2022).
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The 15th Minipig Research Forum

Registration is open!

10-12 May 2023 | Amsterdam, the Netherlands

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. Take part in this conference packed with scientific lectures, poster presentations and the opportunity of networking with minipig users from all over the world.

DATE AND REGISTRATION

Starts	**10 May 2023** at 15:00 hrs CEST (Registration desk opens at 14:00 hrs CEST)
Ends	**12 May 2023 (Friday)** at 12:30 hrs CEST
Registration	**€400** (early bird before 15 February 2023) Late registration fee: €500

The registration fee covers keynote presentations, four scientific sessions, one breakout session of choice, catering (lunch, coffee and snacks), get-together dinner Wednesday evening incl. drinks and networking, dinner Thursday evening, online access to conference material.

The MRF is one of my favourite 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.

SCIENTIFIC SESSIONS

- **Keynote presentation: Introducing the Göttingen micropig**
- **Göttingen Minipigs used in advanced therapeutics**
  The use of humanized IgG Göttingen Minipigs, novel modalities, etc.
- **Biomarkers in Göttingen Minipigs**
  What is in the toolbox/commercially available now?
- **Animal welfare - next level**
  Introduction of the Marseille Declaration, topics relating to training and welfare, etc.
- **Göttingen Minipigs in drug formulation development**
  Perspectives on vehicles, excipients, and routes of administration

Each session will contain four presentations by speakers presenting the latest data and knowledge within their specialist areas. The speakers and presentations will be announced together with the full scientific program, which will be available at minipigresearchforum.org during February 2023.

MORE INFORMATION

For more information on how to submit a poster, about the breakout sessions, and venue, please visit minipigresearchforum.org, where you can also apply for membership.

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). The MRF conference requires physical attendance from the attendees. Read more and apply for membership at minipigresearchforum.org

Developing a perinatal asphyxia model with newborn Göttingen Minipigs at the Ellegaard Göttingen Minipigs A/S research facility

ABOUT STUDY INSIGHTS: The neonatal Göttingen Minipig represents a valuable model for preclinical studies, as it shares a striking number of developmental similarities with human neonates[1-5]. Since the neonatal piglet is a well-established model for perinatal asphyxia, the neonatal Göttingen Minipig was explored for asphyxia, under cooling conditions. This section aims to provide an insight into the development of disease model in neonatal Göttingen Minipigs, in view of translational research.

Insight provided by:
Marina-Stefania Stroe and supervisors Lieselotte Van Bockstal and Steven Van Cruchten | Comparative Perinatal Development (CoPeD), University of Antwerp, Antwerp, Belgium.

What is the study about?

Perinatal asphyxia (PA) is a medical condition that affects 2 to 10 per 1 000 term babies globally, of which approximately one million die. It is caused by oxygen deprivation, which can occur in utero, during labour or delivery, and is the leading cause for severe neurological conditions, such as hypoxic ischemic encephalopathy (HIE). No specific treatment is available at the moment, except for therapeutic hypothermia (TH) [i.e. core body temperature (T°) of 33.5°C for 72 h][6]. In these cases, intensive care unit admission with multiple drug therapies (sedatives, antibiotics, analgesics) is needed[7]. We hypothesize that cooling has an impact on the pharmacokinetics of the administered drugs, potentially leading to higher drug exposure in these patients. For the aim of this project, our research team selected the neonatal Göttingen Minipig, as the animal model for in vivo and in vitro studies.

What is the purpose of the study?

The purpose of this in vivo work was to explore the neonatal Göttingen Minipig for gaining more insight in the impact of hypoxia and hypothermia on the pharmacokinetics (PK) of several commonly used drugs in the neonatal intensive care unit (NICU): fentanyl, midazolam, topiramate and phenobarbital. The main goal of this project is to combine in vitro and in vivo tools with physiology-based pharmacokinetic (PBPK) modelling to support precision dosing in asphyxiated neonates, undergoing cooling[6].

Image 1
Hypothermia management in anaesthetized newborn Göttingen Minipigs. Metallic foil and electric heating blankets were used underneath and to cover the neonatal minipigs reducing radiant, conductive and convective heat losses. The judiciously use of positive pressure ventilation (IPPV) and heat moisture exchangers (HMEs) were strategies considered for body temperature control.

Why is it important?

This project will advance science in the field of paediatric pharmacotherapy and thermo-pharmacology and will also benefit society by improving therapeutic strategies of very ill human neonates, reducing the neonatal morbidity and mortality. The multidisciplinary nature of the project will allow the development of a new tool, the neonatal hypothermia PBPK framework, a novel concept and future approach into addressing important health issues.

What makes this study particularly interesting?

Working at Ellegaard Göttingen Minipigs A/S was an immersive and captivating experience. Investigating the neonatal Göttingen Minipig for perinatal asphyxia felt like pushing the limits of science, since this project aims to benefit society by improving therapeutic strategies of very ill human neonates. Sometimes it was challenging due to the complexity of the model and the small size of the animals, but these hurdles were solved by an adequate stabilizing period, appropriate use of anaesthetic medications, close monitoring and refined surgical techniques. The team was inspirational with their ability to communicate, fast problem solving, efficiency and empathy in the critical moments. Specially for this study, when very specific consumables and intensive care devices were needed, everyone was willing to help and provide everything needed to achieve our goal.

High animal welfare is a prerequisite for high scientific quality. The 3Rs (Replacement, Reduction and Refinement) (Russell and Burch 1959) in relation to animal experimentation is widely accepted as ethical framework for the use of the laboratory animals. The implementation of the 3Rs was also considered for our study, and alternative methods will be performed, in the further steps of the project. Developing a PBPK framework is the main goal of this

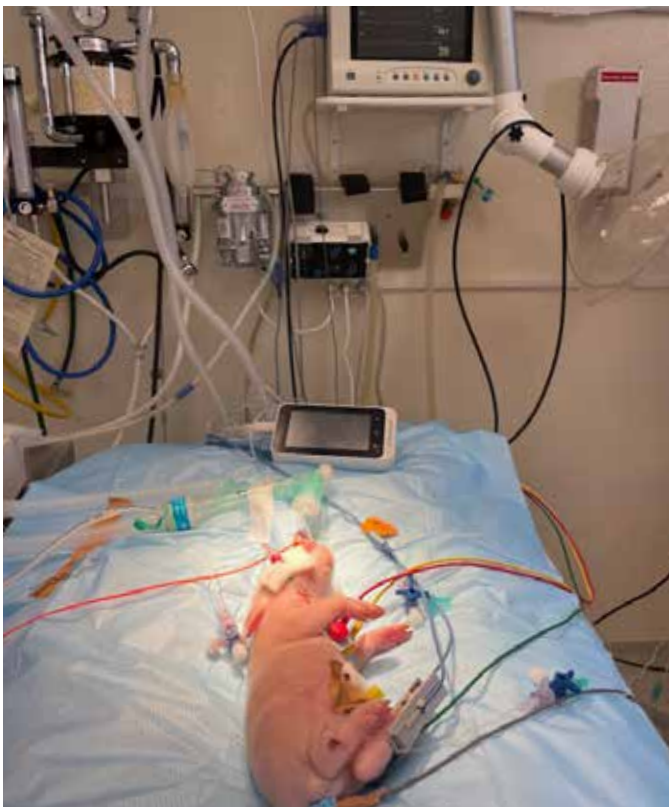

Image 2
Monitoring and venous access in newborn Göttingen Minipigs. The plane of anaesthesia, heart rate, oxygen saturation, carbon dioxide expired, and body temperature were easily obtained and monitored. Central venous catheterisation has been shown to be the main method of vascular access, either for sampling or drug administration. The most common site was the neck, where the external jugular vein was catheterised.

project and represents an in silico model. This can provide important replacement and reduction in the future. However, this cannot be developed without in vivo data, to validate the predicted PK.

The experimental animals were always under anaesthesia, with proper analgesia for the invasive procedures. Also, the human endpoints were designed from the beginning of the study and euthanasia was performed in the most humane manner. The neonatal Göttingen Minipigs, after they suckled sufficient colostrum and within 24h of birth, were selected in the barrier and transferred to the preparation room, by respecting the biosecurity rules. After their admission, they were immediately sedated and anaesthetized to diminish the stress. Close monitoring for maintaining a stable physiological state was achieved to minimize data variability and increase study power. Both contributed to the reduction of the number of animals required in the project. The 3Rs are a good example of how ethical and social concerns have been safeguarded in the present project, matching the core values of Ellegaard Göttingen Minipigs A/S.

Which challenges have you met during the study?
There are some limitations of this model for neonatal asphyxia. The side effects, related to anaesthesia and surgical trauma stress, limited this model in terms of survival length to 24-48h and number of invasive procedures performed over one experimental design. These may be minimized with an adequate stabilizing period, appropriate use of anaesthetic medications, refined surgical techniques, as well as the inclusion of sham-operated control animals for comparison[8]. The techniques used for experimentation in neonatal minipigs require experience from the investigator and his/her assistant, who must be comfortable with both the surgery and anaesthesia. A fully supplied operating theatre is required, particularly for survival and prolonged experiments[9].

How do you recommend going about species selection?
The selection of the optimal juvenile animal model is of utmost importance. From previous research in our group, we know that drug metabolism in developing Göttingen Minipigs shows many similarities with the paediatric population[3-5]. As the conventional pig is a well-established model for perinatal asphyxia and the Göttingen Minipig is the most used pig strain in nonclinical drug development, we wanted to explore this model for potential further use in paediatric drug development programmes.

Any learnings you would like to share??
The logistics of getting neonatal piglets in a research laboratory is a key component of the success of the study. This is a delicate aspect since the transportation of neonatal laboratory animals is prohibited. In our case, we decided to perform the experiments at the Ellegaard Göttingen Minipigs A/S research facility, as this ensured scientific validity and consistency, fair subject selection and randomization to the target groups, reducing stress of the animals and respecting the animal welfare. Ellegaard Göttingen Minipigs A/S gave us the possibility and helped in transforming this research study in a collaborative and feasible achievement, with the scope to develop and validate the neonatal Göttingen Minipig as a new translational animal model.

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Spotlights

VAB/VAP pre-implants in Göttingen Minipigs

Superficial vessels in the minipig are few and frequent access for infusion or blood sampling can be a challenge. Restraint and venepuncture can be stressful and affect blood parameters and therefore catheterization is often the recommended solution for repeated access.

Get an overview the benefits of pre-implanted Vascular Access Buttons vs. Vascular Access Ports here: bit.ly/VAB-VAPimplants.

Telemetric devices in Göttingen Minipigs

Collecting arterial blood pressure, ECG data and eft ventricular data is preferably done from undisturbed animals. Therefore, implanted telemetric devices transmitting data wirelessly to a remote receiver is much preferred. However, the implantation procedure is not easy, which is why you can now have your Göttingen Minipigs with pre-implanted telemetric transmitters, ready for use.

Get more information on pre-implanted telemetric devices in Göttingen Minipigs here: bit.ly/telemetry-GM.

Mixing Göttingen Minipigs across pens

Göttingen Minipigs are social and gregarious animals, and it is therefore recommended that they are housed with others of their kind. Animal Caretaker at Ellegaard Göttingen Minipigs, Tania Panfilova, shares her experience:

"Males and females should always be housed separately following weaning. In general, females have a more docile temper than males, and it is therefore easier to mix them across pens and can be done at all ages. Males, on the other hand, become increasingly aggressive towards new pen mates with age and we therefore only mix across pens until 6 months of age. To help distract the minipigs during the adaptation and smooth the transition, we make sure plenty of enrichment is available such as bedding, ice blocks, toys, food pellets scattered on the floor, etc. to stimulate their senses. Also, we have observed that mixing neighbouring pens have proven less violent when establishing the new hierarchy. Newly mixed pens are always monitored closely so we can intervene in case of prolonged or severe fighting."

Health Monitoring Report: December 2022

Every 6 months the Health Monitoring Report (HMR), based on FELASA recommendations, is published for all three barriers at Ellegaard Göttingen Minipigs.

We are pleased that the health status of the minipigs is continuously high. This year, a single pig tested positive for a new agent in our test panel, Giardia. However, repeated re-testing has not been able to confirm this finding and our overall assessment is that this finding is of limited importance. Our Principal Laboratory Animal Veterinarian, Maja Ramløse, is of course available for potential follow up questions on this matter. Contact her directly at mra@minipigs.dk.

As usual, an exclusion list alongside our HMR is reported to give a quick overview of which agents prompt either authority notification or further diagnostic or corrective action.

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

How to click train Göttingen Minipigs

Previously, several Animal Caretakers from Ellegaard Göttingen Minipigs has attended a clicker training courses at Copenhagen Zoo. In the fall of 2022, another 10 Animal Caretakers was enrolled in an extensive animal training course led by Associate Professor Dorte Bratbo Sørensen, Ph.D., a leading specialist in the field of animal training. The course stretched over two days and focused particularly on clicker training.

"At Ellegaard Göttingen Minipigs we strive to always be better than yesterday, and this includes continued professional development and training. This is also reflected in our dedication to Culture of Care and to upholding our core values; Animal welfare, quality, respect and collaboration", says Carina Anker, Animal Welfare Technician at Ellegaard Göttingen Minipigs A/S and elaborates: "By learning how to train our minipigs in the best possible manner and in our own settings, we can ease animal handling and thereby increase animal well-being. The knowledge gained at this course allows us to better understand our animals and notice the signals they send."

Training the minipigs is an important approach in animal welfare as it creates a level of communication between animal and caretaker, hence resulting in calmer behaviour during handling, which ultimately also increases job satisfaction for the Animal Caretaker. It is even possible to pre-train the minipigs according to specific client needs, such as following a target stick, walking on a treadmill, being milked by hand, etc.

For questions regarding training of Göttingen Minipigs, please contact Laboratory Animal Research Veterinarian, Susi Søgaard, at sso@minipigs.dk.

NEWS FROM

Ellegaard Göttingen Minipigs A/S

Ellegaard Göttingen Minipigs A/S is a leading international company supplying Göttingen Minipigs for biomedical research around the world. From our AAALAC accredited facility in Denmark we breed Göttingen Minipigs and enable the development of safer and more effective medicines, all based on our core values: Animal welfare, quality, respect, and collaboration.

New appointments

Martin Windfeld Velin was appointed Chief Executive Officer on 1 October 2022.

Martin recently served as COO at Ellegaard Göttingen Minipigs and will leverage his overall experience to guide the organisation through its next ambitious phase of growth.

Søren Vangsgaard joined as new Head of Operations on 1 December 2022.

Søren has almost 20 years of experience from Ellegaard Göttingen Minipigs, and after a brief employment outside the organisation Søren has now returned in his new role.

Brian Christiansen joined as new Chief Financial Officer on 1 January 2023.

Brian has more than 20 years of financial experience from international organisations, most recently as Finance Manager at Emerson Automation Solutions.

Christmas sponsorships

Every year at Christmas, Ellegaard Göttingen Minipigs makes donations to a selection of charities. The size of the donations is decided through an internal vote amongst all employees, with the aim of creating ownership and unity.

The charities are selected to support the organisational focuses on corporate values, purpose, or UN Global Goals. This Christmas the recipients of the Christmas donations were:

- Danish Cancer Society and Danish Hospital Clowns, supporting the purpose of enabling the development of safer and more effective medicines.
- Animal Protection Denmark, supporting the core value "Animal Welfare".
- Forests of the World and SOS Children's Villages, supporting the UN Global Goals for Sustainable Development.

UN Global Goals initiative

With the aim of reducing the carbon footprint which is a natural result of the organisation's operation, Ellegaard Göttingen Minipigs has planted 808 trees as part of a climate forest in Denmark. During the lifetime of the trees, they will absorb carbon dioxide corresponding to 202 tons CO2.

The forest also ensures and enriches biodiversity, which is another area Ellegaard Göttingen Minipigs has been focusing on working with the UN Global Goals for Sustainable Development.

With this initiative, Ellegaard Göttingen Minipigs becomes a carbon neutral organisation in 2023.

Where to meet us in 2023

CONFERENCE	DATE	LOCATION
International Pain Conference: Bench to Bedside	8-9 Mar	Ness Ziona, Israel
SOT and ToxExpo	19-23 Mar	Nashville, Tennessee, USA
IAT Congress	21-24 Mar	United Kingdom
Janssen Juvenile Tox Symposium	20-21 Apr	Beerse, Belgium
Scand-LAS	25-28 Apr	Uppsala, Sweden
AFSTAL	7-9 Jun	Bordeaux, France
GV-Solas	6-8 Sep	Mainz, Germany
EUROTOX	10-13 Sep	Ljubljana, Slovenia
SPS	18-21 Sep	Brussels, Belgium
LASACON	TBA	TBA
STP-I	TBA	TBA

INTRODUCING

GÖTTINGEN MINIPIGS ACADEMY

Ellegaard Göttingen Minipigs is all for sharing and believe that openness creates trust, enriches, and clears the path for new opportunities. ***We create fora for networking and knowledge sharing*** *about Göttingen Minipigs in biomedical research.* ***We support scientific research*** *through our Research Foundation.* ***And we educate*** *through the Göttingen Minipigs Academy.*

To whom is the academy addressed?

The academy is a new initiative that facilitates seminars and workshops on various topics concerning Göttingen Minipigs, targeted at those working within the life science industry.

In addition to the prescheduled courses, we also offer on-demand lectures and hands-on training tailored to your specific needs and interests. Training can be carried out at the premises of Ellegaard Göttingen Minipigs A/S in our well-equipped seminar room, or in our animal and laboratory facilities, but can also be conducted at your own or a remote site.

What can be expected from the Academy?

The Göttingen Minipigs Academy will present well-organised courses with a high level of knowledge sharing based on a practical, theoretical, scientific, or hands-on approach, and at all times conveyed by experts within their respective fields.

Through the Göttingen Minipigs Academy, you will gain access to:

- Hands-on training
- Expert lectures
- On-demand courses
- Webinars
- Printed materials
- Networking opportunities

How and when can I register?

The courses will be open for registration in Spring 2023. Follow us on LinkedIn, where we will announce new courses on an on-going bases or keep an eye on minipigs.dk/events.

Open house on 14 December 2022

The very first Göttingen Minipigs Academy course was conducted in December with participants from Denmark and the UK.

First, the participants got a tour of the premises including a visit to the viewing lofts. From here you can see the Göttingen Minipigs in their pens through a window in the floor. Next, Laboratory Technician Adrian Zeltner, SRS, demonstrated handling and catheterization of Göttingen Minipigs in our laboratory, and the participants had the chance to place different catheters in an anesthetized minipig.

Finally, a theoretical lecture explained the origin and nature of Göttingen Minipigs and how we ensure animal welfare with a dedicated enrichment program at Ellegaard Göttingen Minipigs.

Contact us

If you have questions about the content of the courses or the Academy in general, please contact Laboratory Animal Research Veterinarian and Academy Facilitator, Susi Søgaard at sso@minipigs.dk.

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.

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