No. 67 | Winter 2024

Göttingen Minipigs MAGAZINE

SPECIAL EDITION

Juvenile Göttingen Minipigs in Biomedical Research

ELLEGAARD
GÖTTINGEN MINIPIGS

Dear reader

2024 is already well underway. Time moves fast, and soon we will be attending this year's conferences once again to shake hands, catch up, and discuss new opportunities. Across the US and Europe, we will be present at around 20 conferences and tradeshows, starting with SOT and ToxExpo 2024 in Salt Lake City. In the autumn, EUROTOX 2024 will take place in Copenhagen and present an opportunity to visit our facility on the Sunday before the conference. You can see the full list of conferences where you can meet us on page 23.

One conference that stands out is naturally the 16th Minipig Research Forum taking place 22-24 May 2024. The scientific

CONTACT

Ellegaard Göttingen Minipigs A/S

Soroe Landevej 302
4261 Dalmose
Denmark

+45 5818 5818

ellegaard@minipigs.dk

www.minipigs.dk

programme has been made public and I recommend that you take a look at it on page 29.

You can also expand your knowledge of Göttingen Minipigs in biomedical research through the Göttingen Minipigs Academy. The courses have been carefully selected to accommodate different needs depending on profession, and target both scientists and those working practically with Göttingen Minipigs. You can see the full course calendar on pages 26-27.

In this edition of the magazine we are trying something new:
A special edition on the theme "use of juvenile Göttingen Minipigs in biomedical research". The aim is to provide a glimpse into one more particular area of study and how much material is available. You will find a selection of articles published in the magazine over recent years, a webinar recording, a special reference list, and some interesting insights on the topic. I hope you will find it both useful and inspiring.

While this edition is focused on a specific theme, the editorial article on the following pages takes a different, yet contemporary and relevant, approach. It presents the ESG profile of Ellegaard Göttingen Minipigs A/S and shares information about our company's environmental approach, social responsibility, and governance policies.

I hope you enjoy this themed edition.

Martin Windfeld Velin, CEO
Ellegaard Göttingen Minipigs A/S

CONTENTS

ESG: A vision, a plan, a promise

Around the world organisations are scrutinising their routines, production set-up, and emission levels trying to find sustainable yet profitable alternatives, so they can contribute to the UN Global Goals, meet the growing demands of corporate ESG profiles, and create a better and more sustainable world.

Ellegaard Göttingen Minipigs is an example of a company that has grown and expanded its facilities over the years. And as they have done so, sustainable and energy-saving solutions have always been a priority, for example by choosing a heating solution based on geothermal heat supported by a straw-fired boiler. Søren Vangsgaard, Head of Operations, explains the setup: "The temperature in our breeding barriers is regulated through geothermal heat. Two meters underground there is a constant temperature of 8 °C. This temperature heats or cools the air in the barriers as it passes through approx. 30 km of underground pipes. The 8 °C cold air means that we use no electricity on air-conditioning systems in the summer, and in the winter the temperature can likewise be raised to 8 °C. Of course, 8 °C is too cold for indoor conditions, so instead of using fuel oil we have a supporting straw-fired boiler to heat our entire facility, both breeding barriers and office buildings, and heat our water. This solution saves at least 70,000 litres of fuel oil every year and classifies our heating solution as carbon neutral. Furthermore, the straw comes from the surrounding fields and is this way part of the local eco-system."

Adding to this, Ellegaard Göttingen Minipigs has installed 8 charging stations supporting employees to choose electric cars, and is currently testing electric cars for local deliveries as well.

Other initiatives - big and small

Not all changes have to be expensive, long-term investments such as solar panels and geothermal heat. Here are examples of other initiatives taken at Ellegaard Göttingen Minipigs that contributes to reduced emission and environmentally friendly consumption:

- Planting of 808 trees as part of a climate forest in Denmark, which will absorb carbon dioxide corresponding to 202 tons CO2 during the trees' lifetime and ensure and enrich biodiversity
- Meticulous sorting of waste and garbage incl. correct handling of hazardous and medical waste
- Limiting fuel consumption and emission by offering the opportunity to work from home
- Purchasing of supplies is accumulated to limit transport emissions
- Digital routines limiting printing and the use of paper
- Distributing the Göttingen Minipigs Magazine electronically, and the few hard copies are printed on FSC certified paper marked with The Nordic Eco-label
- Only tap water is served
- Close monitoring of power and water consumption, making it easier to discover malfunctions and prevent overconsumption
- Replacement of light bulbs and fluorescent lamps with LED solutions
- Automatic light control in administration building dimming the lights when there is no activity

Model of geothermal heat solution with underground pipes.

Locally grown straw for the straw-fired boiler.

Last but not least, the company has installed solar panels that will produce approx. 150,000 kWh corresponding to 25% of the company's total power consumption. "The investment in solar panels has been discussed for a while, as it holds great potential for limiting our power expenses, but not least comes as a natural next step in our focus on sustainable development" CEO, Martin Windfeld Velin, explains.

The social aspect of the ESG profile is equally important at Ellegaard Göttingen Minipigs. A high level of animal welfare has always been the company's utmost priority, as is creating a healthy work environment for all employees and ensuring access to relevant training and education. Martin Windfeld Velin says: "We wish our employees to keep evolving within their respective fields of expertise through continued education, so we can maintain high quality in our services and collaboration with our customers. But it is also very much in our interest that our employees feel engaged, valued, and supported in their development." To support a healthy work environment, Ellegaard Göttingen Minipigs has also conducted internal workshops as part of the focus on creating and maintaining a good Culture of Care, incl. compassion fatigue.

Supporting the internal social priority, Ellegaard Göttingen Minipigs' likewise takes on their corporate social responsibility. 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, to create engagement and unity. The charities are selected to support the organisational focus on corporate values, corporate purpose, and UN Global Goals. For 2023, the recipients of the Christmas donations were:

- Supporting the corporate purpose of enabling development of safer and more effective medicines: Danish Cancer Society and Danish Hospital Clowns
- Supporting the core values of Animal Welfare and Respect: Animal Protection Denmark
- Supporting the UN Global Goals for Sustainable Development: Forests of the World and SOS Children's Villages

To accommodate governance requirements, Ellegaard Göttingen Minipigs works continuously with compliance. This is not a new initiative but has always been part of the company's good business practices and commitment to conduct business with the highest standards of ethics and integrity. As examples can be mentioned the Standard Operating Procedures (SOP), which serve to ensure and document reliability, consistency, and high-quality standards. Most recently Ellegaard Göttingen Minipigs has upgraded their whistle-blower solution and finalised an official document stating their code of conduct.

Questions concerning Ellegaard Göttingen Minipigs' ESG profile can be forwarded to ellegaard@minipigs.dk.

2 of the 8 charging stations for electric cars.

Solar panels at Ellegaard Göttingen Minipigs A/S.

The use of neonatal and juvenile (mini)pigs in drug discovery and drug development

By Miriam Ayuso and Steven Van Cruchten[1]

[1]Comparative Perinatal Development (CoPeD), Department of Veterinary Sciences, University of Antwerp, Belgium

Drug therapy in paediatric patients is challenging in view of the maturation of organ systems and processes that affect pharmacokinetics (PK) and pharmacodynamics. Especially for the youngest age groups and for paediatric-only indications, neonatal and juvenile animal models can be useful to assess drug safety and to better understand the mechanisms of diseases or conditions. In this respect, the use of neonatal and juvenile pigs in the field of paediatric drug discovery and drug development is promising, although still limited at this point.

We summarized the comparative postnatal development of pigs and humans and discussed the advantages of the neonatal and juvenile pig in view of developmental pharmacology, paediatric diseases, drug discovery and drug safety testing in the following review paper: Ayuso, M.; Buyssens, L.; Stroe, M.; Valenzuela, A.; Allegaert, K.; Smits, A.; Annaert, P.; Mulder, A.; Carpentier, S.; Van Ginneken, C.; Van Cruchten, S. The Neonatal and Juvenile Pig in Pediatric Drug Discovery and Development. Pharmaceutics 2021, 13, 44. https://doi.org/10.3390/pharmaceutics13010044 [1]. For a complete overview, we refer to this open access paper. In the following paragraphs, we will only discuss the highlights.

Nonclinical *in vivo* models, like the neonatal and juvenile pig are of increasing interest in paediatric drug development from two perspectives. First, to investigate and consequently better understand the mechanism of a disease, particularly when it is unique to paediatric patients. Second, the model may also provide important safety data for the paediatric population when performing juvenile toxicity studies. The choice of species and the design of juvenile toxicity studies are therefore the result of a series of complex considerations, including the therapeutic use of the drug, the age at which children will be treated, the duration of treatment, and potential age- or species-specific differences in efficacy, PK, or toxicity observed in adult animals.

Reprint notice

Originally published in the Göttingen Minipigs Magazine #60, Spring edition 2021.

The utility of a 'leverage concept' for dose determination and drug development programs in neonates has recently been described [2]. The following scenarios can be distinguished:

- Paediatric disease similar to that in adults and/or older paediatric patients where dosing is known for adult and/or older paediatric patients = extrapolation of efficacy from adults to paediatric patients is permitted, and even supported.

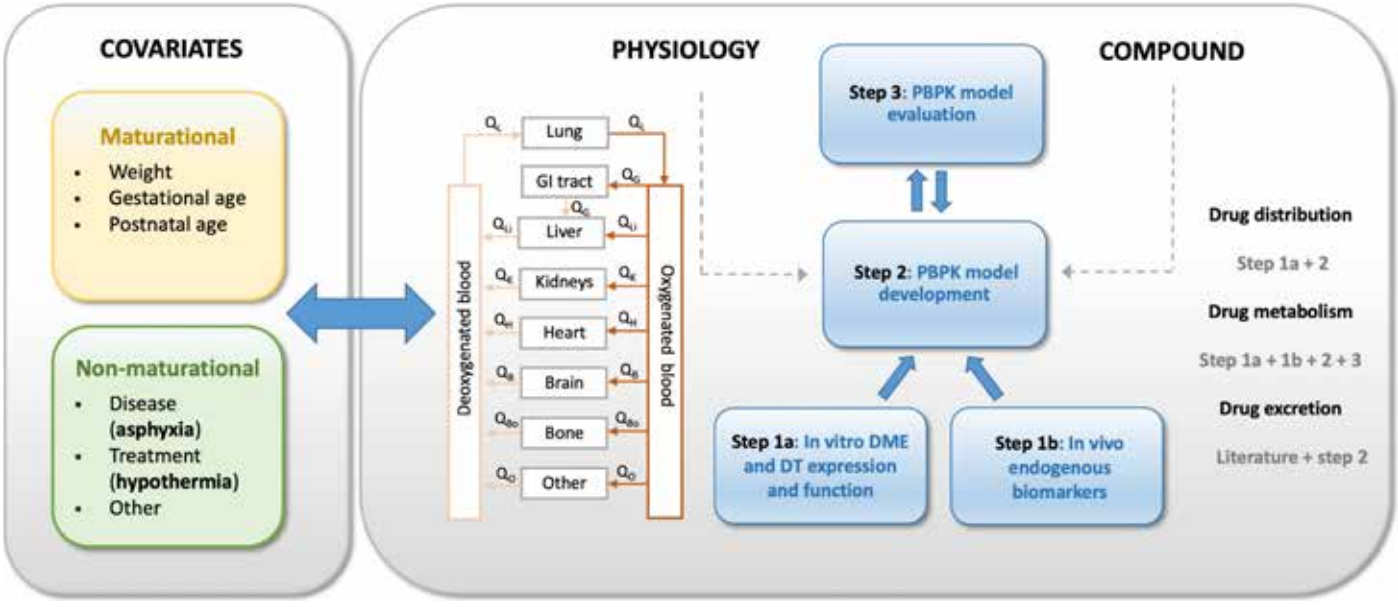


Figure 1
Strategy for a neonatal hypothermia physiology based pharmacokinetic (PBPK) framework development. DME: drug metabolizing enzymes; DT: drug transporters. Adapted from [8].

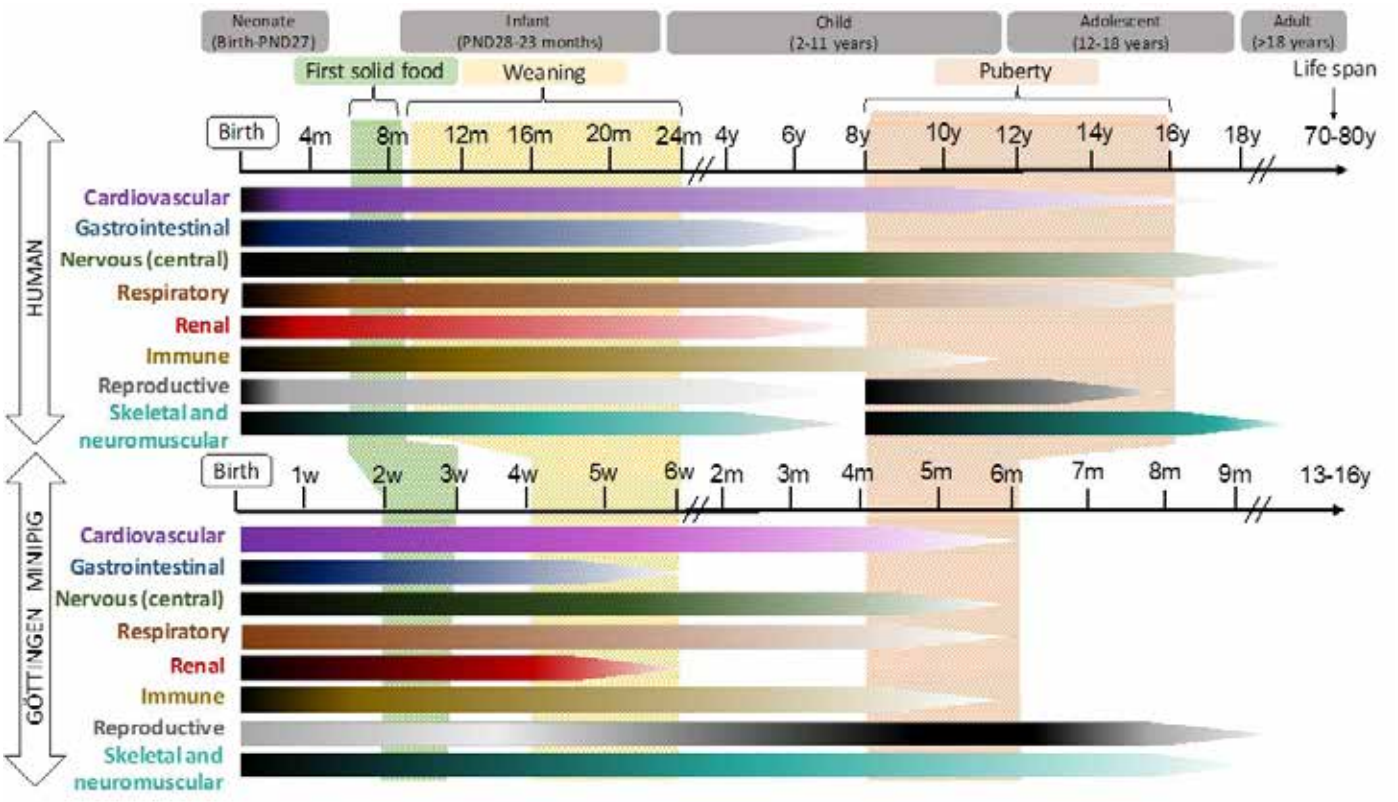


Figure 2
Schematic representation of the postnatal development of different organ systems in human (top) and Göttingen Minipig [1]. In the horizontal bars, the intensity of the maturation process is represented by dark (more intense) and light (less intense) tones. The time bar represents weeks (w), months (m) or years (y) of life.

- Paediatric disease related but not similar to that in adults and/or older paediatric patients where dosing is known for adult and/or older paediatric patients = additional information can be leveraged from either in vitro or *in vivo* models to guide initial dosing.
- Paediatric disease unique to a given (sub)population within paediatrics, where these drugs are not utilized for these specific diseases in adults.

Even in the setting of similarity, additional research in juvenile animals may still be warranted when concerns related to developmental toxicology (like growth, neurodevelopment, kidney, or cardiovascular system) should be addressed. Only about 10% of the 400 products (almost exclusive new drug approvals) of which the labels were reviewed between 1998–2009 by the FDA contained information on juvenile animals [3]. In a recent survey on European Paediatric Investigation Plan (PIP) decisions (2007–2017, 229 drugs) with juvenile animal requests, general toxicological studies were the most applicable study designs, with infectious diseases, endocrinology, neurology, and cardiovascular diseases being the most common therapeutic areas. As anticipated, about 80% of these studies were in rats, while studies in pigs were limited (4.2%) [4]. Interestingly, a recent European Medicines Agency (EMA) analysis on juvenile animal studies in the field of anticancer drug research documented that juvenile models also generated evidence regarding new target organ toxicity (kidney, central and peripheral nervous system, impaired learning or memory, cardiac system) or increased severity of toxicity (including mortality rate) [5]. At the other end of the spectrum, with diseases that are unique to a given subpopulation within paediatrics, pig models can be instrumental in drug discovery and development for accurate mechanistic understanding of the disease or condition. Specific to neonates, this has been described for, e.g., necrotizing enterocolitis (NEC), resuscitation practices, or perinatal asphyxia. Studies in pigs have established the essential roles of prematurity, microbial colonization, and enteral nutrition in the pathogenesis of NEC [6]. The (juvenile) pig is also an important animal model in research on human resuscitation [7]. In addition, *in vivo* data generated in neonatal animals—including (mini)pig—facilitate the development of a neonatal physiology-based PK (PBPK) model during therapeutic hypothermia [8] (see Figure 1).

In order to assess the feasibility of the neonatal and juvenile pig as models for paediatric drug development, an in-depth characterization of this model must be carried out in the first place, followed by a comparison of the anatomical, physiological and ADME characteristics in the corresponding paediatric age groups.

Regarding anatomical and physiological characteristics, our group has already reported on the age-related maturation of organ weights in the developing Göttingen Minipigs in an effort to further develop a PBPK model [9], but more data are needed. The implementation of this model would benefit from data on microsomal protein per gram of liver and abundance on drug metabolizing enzymes during development, or from a better

understanding of pig orthologues for human cytochrome P450 (CYP) enzymes. For the developing domestic pig, the anatomy, physiology and the absorption, distribution and excretion of drugs have been reviewed by others [10]. As some of the above data are publicly accessible in the ICH S11 guideline on nonclinical safety testing in support of the development of paediatric pharmaceuticals [11], we will only highlight some key points. The EMA has established different age categories within the paediatric population, and many similarities between human and Göttingen Minipigs organ development (as the reference breed used in the pharmaceutical industry) were reported in the ICH S11 guideline. In general, pigs and humans share many developmental milestones: The patterns of development of the gastrointestinal tract (GIT), the cardiovascular, the CNS systems and the eye are quite similar in both species, while renal, immune, and reproductive development occur slightly earlier and more quickly in humans than in pigs. These data are illustrated in Figure 2.

Regarding ADME characteristics, hepatic Phase I drug metabolism mediated by CYP enzymes has been investigated extensively in adult conventional pig strains and minipig strains over the past 30 years. Knowledge on the ontogeny of these processes in the neonatal and juvenile population is much more limited. Particularly in neonates, it is crucial to predict drug disposition correctly in order to avoid inefficacy due to underdosing or adverse effects caused by overdosing. Recently, CYP activity was determined in neonatal and juvenile conventional pig [12] and Göttingen Minipigs [13] in different age groups using several human CYP450 substrates. As such, substrate specificity was examined and CYP450 activity levels in (mini)pig were compared to those in human. In Göttingen Minipigs, we found that CYP450 enzyme activity increased postnatally. However, differences in onset and speed in development were observed: CYP1A2- and CYP2D6-like activity levels increased fast during the first week of life, whereas CYP2C9- and CYP3A4-like activities matured more slowly, reaching their highest levels in 1-month-old pigs [13], corresponding roughly to a 2-year-old child (see Figure 3). In the conventional pig, similar results were obtained [12]. In addition, no sex-related differences were observed in the neonatal and juvenile age groups regarding the CYP450 ontogeny patterns until puberty [12,13]. With regard to CYP450 protein abundance, some research has already been conducted in the conventional pig [12], and this question is currently being addressed in Göttingen Minipigs by our group. In general, activity and abundance data correlate well, although

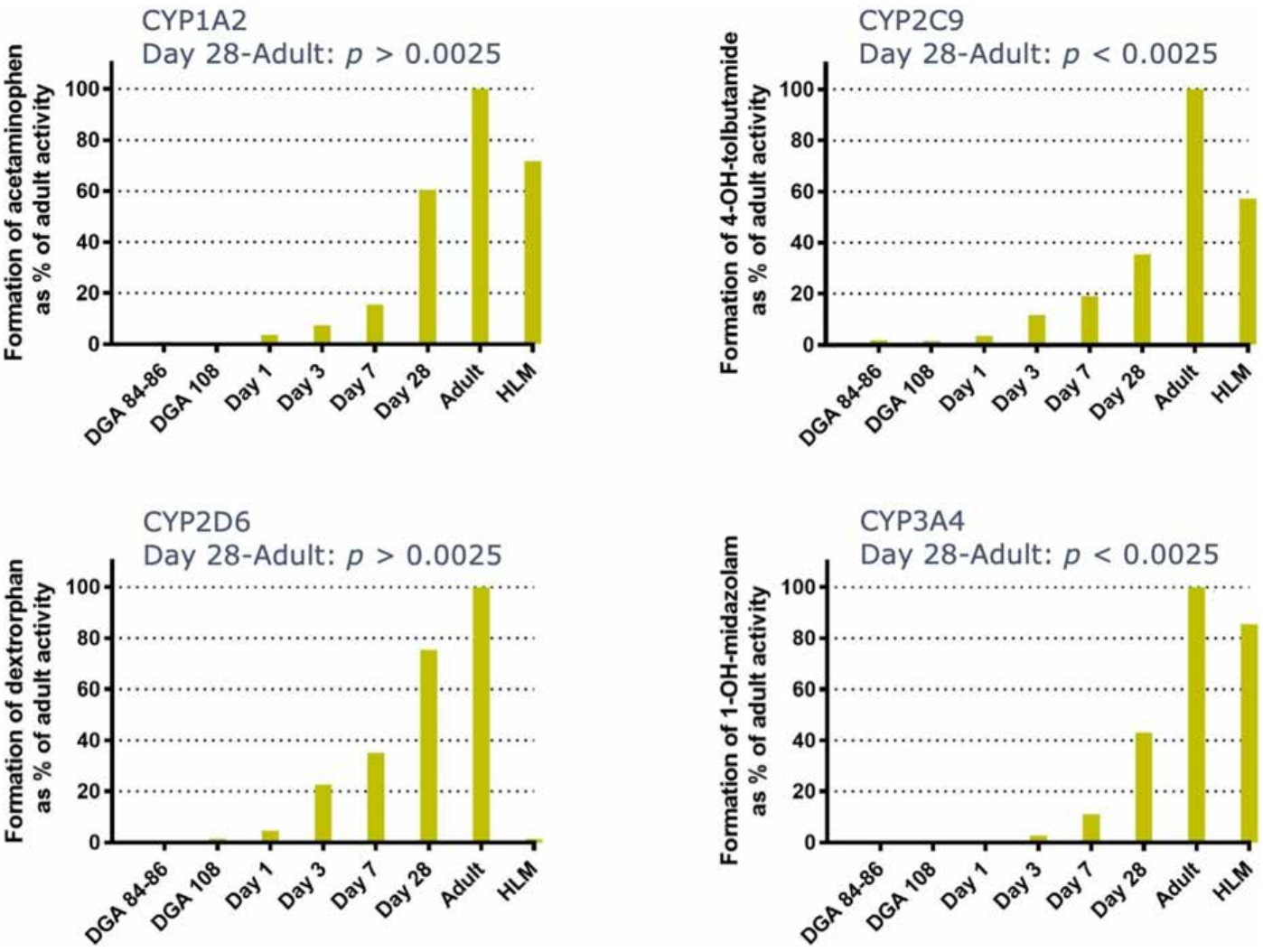


Figure 3
CYP activity profiles in liver microsomes of foetal, neonatal, juvenile, and adult Göttingen Minipigs. DGA: days of gestation; HLM: adult human liver microsomes

CYP isoform-specific differences have been reported [12]. Regarding Phase 2 metabolism, data are scarce. A recent study on 1-day and 2-, 5-, 10- and 20-week old male Camborough-29 pigs showed that in vitro UDP Glucuronosyltransferase (UGT) enzyme activity increased from day 1 until week 10, followed by a decline at around week 20 [14]. An *in vivo* study with ibuprofen in 1-, 4-, and 8-week old, and 6–7-month old, mixed breed pigs also showed UGT activity already in neonatal pigs [15]. In our group, UGT activity was investigated in Göttingen Minipigs with age groups ranging from the late foetal stage until postnatal day 28, and adults [13]. From PND 7 onward, UGT activity increased without sex-related differences and reached adult levels at PND 28. In the youngest age groups (gestational day age (GDA) 84–86, GDA 108, postnatal (PND) day 1 and 3), activities were below the lower detection limit when using a luminescence-based assay. However, immunohistochemical analysis showed that even in the late foetal stages, UGT1A could be detected. In accordance with the activity results, UGT1A detection increased with age [13]. In general, it can be concluded that UGT enzymes are expressed from an early age, but further characterization of the different isoforms is needed in order to better predict drug disposition in this animal model. With regard to drug transport (often referred to as Phase 0 for uptake transporters and Phase III for efflux transporters), the data in the pig are even more scarce. For Göttingen Minipigs, we performed a semiquantitative assessment of P-glycoprotein (P-gp, encoded by the Multidrug Resistance Gene, MDR) in the liver of neonatal and juvenile pigs and foetuses using immunohistochemistry. No difference was observed in P-gp expression between livers from GDA84 to adult animals (1.5–3 years of age) [16].

When comparing the above data with the ontogeny profiles of the drug disposition processes in human, which have been reviewed extensively elsewhere [17-19], remarkable similarities are present. For UGT and P-gp, the ontogeny profile on protein and activity level, if assessed, is very similar. With regard to the CYP activity, the interpretation is more complex. The slow maturation profile of CYP2C9 and CYP3A4 activity in (mini)pigs corresponds well with the paediatric population. For CYP1A2 and CYP2D6, there appears to be an earlier onset of activity in the pig than in human, and CYP2D6 activity in general appears to be much higher than in human. Still, when comparing the pig with man, one needs to be very cautious, as studies may use different substrates or other testing conditions, which may confound the results and, as such, species comparisons. This said, even when not directly translatable to human, in vitro and *in vivo* drug metabolism data in juvenile animals are critical, as they may explain differences in efficacy or toxicity with the human population, and they can be used in PBPK models to better predict exposure, especially in the very young age groups, as further discussed in our review paper.

In conclusion, the physiology and development of several organ systems and conditions associated with (preterm) birth are very similar in pigs and humans. Despite this fact, the use of neonatal and juvenile Göttingen Minipigs, the reference breed in the pharmaceutical industry, in paediatric drug development programs is still very limited. One of the main reasons is the fact that paediatric regulatory guidelines recommend using the same (preferably rodent) species and strain in juvenile animal studies as in adult repeated dose toxicity studies. As regulatory

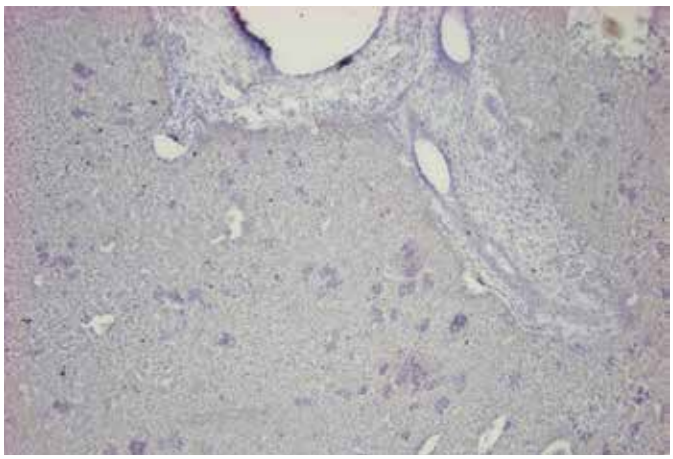


Figure 4a
Immunohistochemical detection of UGT1A in the liver of a Göttingen Minipig fetus at 84-86 days of gestation. A mild staining among all hepatocytes is present. Scale bar 200 µm.

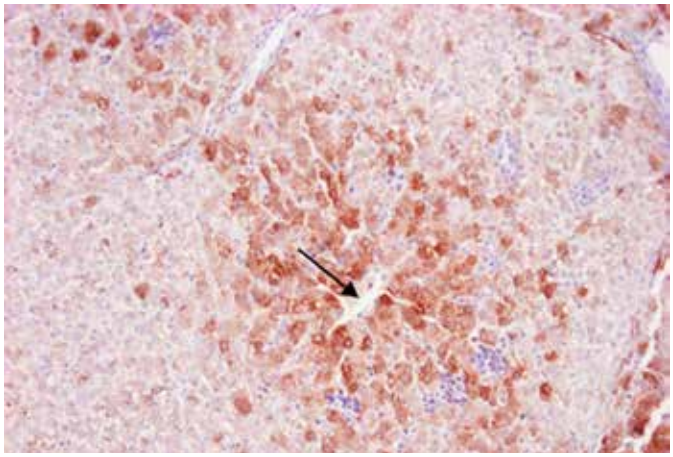


Figure 4b
Immunohistochemical detection of UGT1A in the liver of a 7-day-old Göttingen Minipig. Small groups of more intensively stained hepatocytes appear close to the central vein of each lobule, though not generalized. The black arrow indicates the central vein. Scale bar 200 µm.

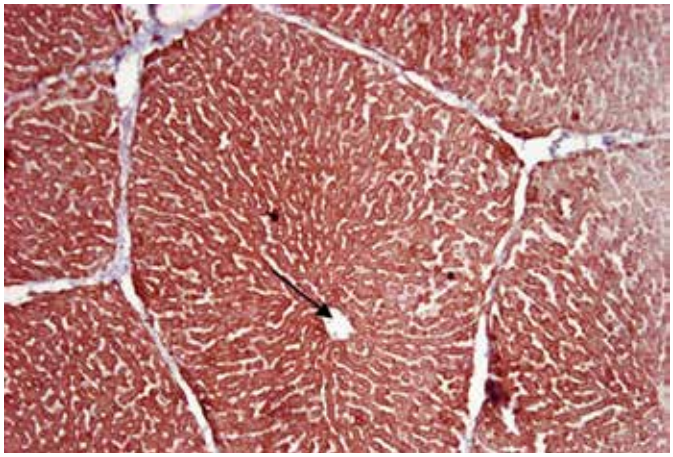


Figure 4c
Immunohistochemical detection of UGT1A in the liver of an adult male Göttingen Minipig. The hepatocytes of the entire liver lobules are intensely stained. The black arrow indicates the central vein. Scale bar 200 µm.

guidelines for adult repeated dose toxicity studies currently do not define species selection in a detailed manner, there is no consistent approach and pharmaceutical companies often base their decision for species selection on experience and data with rats and dogs over the years. As such, Göttingen Minipigs are not considered in their species selection process. In our opinion, including more detailed species selection criteria in the regulatory guidelines for adult repeated dose toxicity studies would increase the use of Göttingen Minipigs in drug safety testing for indications in the adult and paediatric population. For paediatric-only indications, especially in the youngest age groups in which extensive clinical and nonclinical studies in adults are, in general, not performed, pharmaceutical companies should consider by default neonatal and juvenile Göttingen Minipigs for their nonclinical program, as it often represents a better translational model than rat and dog pups. We anticipate that current efforts to fully characterize the model, including ADME processes, and the development of juvenile pig PBPK models will promote the use of the juvenile pig model in paediatric drug safety studies.

REFERENCES

1. Ayuso, M.; Buyssens, L.; Stroe, M.; Valenzuela, A.; Allegaert, K.; Smits, A.; Annaert, P.; Mulder, A.; Carpentier, S.; Van Ginneken, C.; Van Cruchten, S. The Neonatal and Juvenile Pig in Pediatric Drug Discovery and Development. Pharmaceutics 2021, 13, 44, doi:10.3390/pharmaceutics13010044
2. Anker, J.V.D.; McCune, S.; Annaert, P.; Baer, G.R.; Mulugeta, Y.; Abdelrahman, R.; Wu, K.; Krudys, K.M.; Fisher, J.; Slikker, W.; et al. Approaches to Dose Finding in Neonates, Illustrating the Variability between Neonatal Drug Development Programs. Pharmaceutics 2020, 12, 685, doi:10.3390/pharmaceutics12070685.
3. Tassinari, M.S.; Benson, K.; Elayan, I.; Espandiari, P.; Davis-Bruno, K. Juvenile animal studies and pediatric drug development retrospective review: Use in regulatory decisions and labeling. Birth Defects Res. Part B Dev. Reprod. Toxicol. 2011, 92, 261–265, doi:10.1002/bdrb.20304.
4. Baldrick, P. Juvenile Animal Testing: Assessing Need and Use in the Drug Product Label. Ther. Innov. Regul. Sci. 2018, 52, 641–648, doi:10.1177/2168479017750129.
5. European Medicines Agency. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/results-juvenile-animal-studies-jas-impact-anti-cancer-medicine-development-use-children_en.pdf (accessed on 23 October 2020).
6. Burrin, D.; Sangild, P.T.; Stoll, B.; Thymann, T.; Buddington, R.; Marini, J.; Olutoye, O.; Shulman, R.J. Translational Advances in Pediatric Nutrition and Gastroenterology: New Insights from Pig Models. Annu. Rev. Anim. Biosci. 2020, 8, 321–354, doi:10.1146/annurev-animal-020518-115142.
7. Barouxis, D.; Chalkias, A.; Syggelou, A.; Iacovidou, N.; Xanthos, T. Research in human resuscitation: What we learn from animals. J. Matern. Neonatal Med. 2012, 25, 44–46, doi:10.3109/14767058.2012.714633.
8. Smits, A.; Annaert, P.; Van Cruchten, S.; Allegaert, K. A Physiology-Based Pharmacokinetic Framework to Support Drug Development and Dose Precision During Therapeutic Hypothermia in Neonates. Front. Pharmacol. 2020, 11, doi:10.3389/fphar.2020.00587.
9. Van Peer, E.M.; Downes, N.; Casteleyn, C.; Van Ginneken, C.; Weeren, A.; Van Cruchten, S. Organ data from the developing Göttingen minipig: First steps towards a juvenile PBPK model. J. Pharmacokinet. Pharmacodyn. 2015, 43, 179–190, doi:10.1007/s10928-015-9463-8.
10. Gasthuys, E.; Vandecasteele, T.; De Bruyne, P.; Walle, J.V.; De Backer, P.; Cornillie, P.; Devreese, M.; Croubels, S. The potential use of piglets as human pediatric surrogate for preclinical pharmacokinetic and pharmacodynamic drug testing. Curr. Pharm. Des. 2016, 22, 4069–4085.
11. ICH Guideline S11 on Nonclinical Safety Testing in Support of Development of Paediatric Pharmaceuticals; Committee for Medicinal Products for Human Use; 2020.
12. Millecam, J.; De Clerck, L.; Govaert, E.; Devreese, M.; Gasthuys, E.; Schelstraete, W.; Deforce, D.; De Bock, L.; Van Bocxlaer, J.; Sys, S.; et al. The Ontogeny of Cytochrome P450 Enzyme Activity and Protein Abundance in Conventional Pigs in Support of Preclinical Pediatric Drug Research. Front. Pharmacol. 2018, 9, 470, doi:10.3389/fphar.2018.00470.
13. Van Peer, E.M.; Jacobs, F.; Snoeys, J.; Van Houdt, J.; Pijpers, I.; Casteleyn, C.; Van Ginneken, C.; Van Cruchten, S. In vitro Phase I- and Phase II-Drug Metabolism in The Liver of Juvenile and Adult Göttingen Minipigs. Pharm. Res. 2017, 34, 750–764, doi:10.1007/s11095-017-2101-y.
14. Hu, S.X. Age-related change of hepatic uridine diphosphate glucuronosyltransferase and sulfotransferase activities in male chickens and pigs. J. Vet. Pharmacol. Ther. 2016, 40, 270–278, doi:10.1111/jvp.12355.
15. Millecam, J.; De Baere, S.; Croubels, S.; Devreese, M. In Vivo Metabolism of Ibuprofen in Growing Conventional Pigs: A Pharmacokinetic Approach. Front. Pharmacol. 2019, 10, doi:10.3389/fphar.2019.00712.
16. Van Peer, E.M.; Verbueken, E.; Saad, M.; Casteleyn, C.; Van Ginneken, C.J.; Van Cruchten, S. Ontogeny of CYP3A and P-Glycoprotein in the Liver and the Small Intestine of the Göttingen Minipig: An Immunohistochemical Evaluation. Basic Clin. Pharmacol. Toxicol. 2013, 114, 387–394, doi:10.1111/bcpt.12173.
17. Upreti, V.V.; Wahlstrom, J.L. Meta-analysis of hepatic cytochrome P450 ontogeny to underwrite the prediction of pediatric pharmacokinetics using physiologically based pharmacokinetic modeling. J. Clin. Pharmacol. 2015, 56, 266–283, doi:10.1002/jcph.585.
18. Lu, H.; Rosenbaum, S. Developmental Pharmacokinetics in Pediatric Populations. J. Pediatr. Pharmacol. Ther. 2014, 19, 262–276, doi:10.5863/1551-6776-19.4.262.
19. Brouwer, K.L.; Aleksunes, L.M.; Brandys, B.; Giacoia, G.P.; Knipp, G.T.; Lukacova, V.; Meibohm, B.; Nigam, S.K.; Rieder, P.F.M.J.; De Wildt, S.N.; et al. Human Ontogeny of Drug Transporters: Review and Recommendations of the Pediatric Transporter Working Group. Clin. Pharmacol. Ther. 2015, 98, 266–287, doi:10.1002/cpt.176.

Webinar recording:

What's good to know when considering the Göttingen Minipigs for Juvenile toxicology studies?

Webinar by Edward Marsden, Associate Director Charles River Laboratories, Lyon, France.

Abstract | Juvenile animal studies are increasingly being used to support the development of paediatric medicines. Göttingen Minipigs are ideal in many ways for such studies and are specifically mentioned in the close-to-final ICH S11 guideline *[ed.: Legal effective date 26 September 2020]*. Edward Marsden, who has many years of experience in the field, will share his experiences regarding optimizing such studies which are often quite complex.

Recording notice

Originally broadcasted as webinar 30 October 2019.

Scan or click the QR code to watch the recording:

ICH S11 Guideline: Nonclinical Safety Testing for Paediatric Pharmaceutical Development

About the S11 guidelines

The ICH guideline S11 focuses on nonclinical safety testing to support the development of paediatric pharmaceuticals. It was developed by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), which brings together regulatory authorities and the pharmaceutical industry to discuss scientific and technical aspects of drug registration.

The primary objective of ICH S11 is to provide guidance on the appropriate scope and application of nonclinical safety studies to support the safe clinical development of pharmaceuticals intended for use in the paediatric population. This includes considerations for the timing, species selection, design, and execution of nonclinical studies to ensure that they provide meaningful safety information that can be used to cure paediatric patients.

Retrieve the S11 guideline

Go to the EMA website for more information about the ICH S11 guidelines.

Scan or click the QR code for easy access.

About the ICH

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is a global initiative that aims to bring together regulatory authorities and the pharmaceutical industry from different regions to discuss and establish common technical guidelines and standards for the development, registration, and post-approval changes of pharmaceuticals. The goal is to promote public health and ensure that safe, effective, and high-quality medicines are developed and registered in the most efficient and cost-effective manner.

The ICH guidelines serve as a unified standard that member regions (FDA in the United States, EMA in the European Union, and PMDA in Japan) agree to follow, which helps to ensure consistency and reduce the likelihood of major differences between regions, facilitate international collaboration, streamline drug development and approval, and support safe drug development. Member regions participate actively in the ICH process by contributing to the development and update of guidelines and committing to implementing these guidelines within their respective regulatory frameworks. Following the ICH guidelines in the development of a drug, it is therefore more likely to meet the regulatory requirements in all member regions, facilitating the international registration and availability of new drugs, including those intended for paediatric patients.

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.

Reprint notice

Originally published in the Göttingen Minipigs Magazine #59, Winter edition 2020.

Author's comment:
Reference No. 18 has been updated in this reprint.

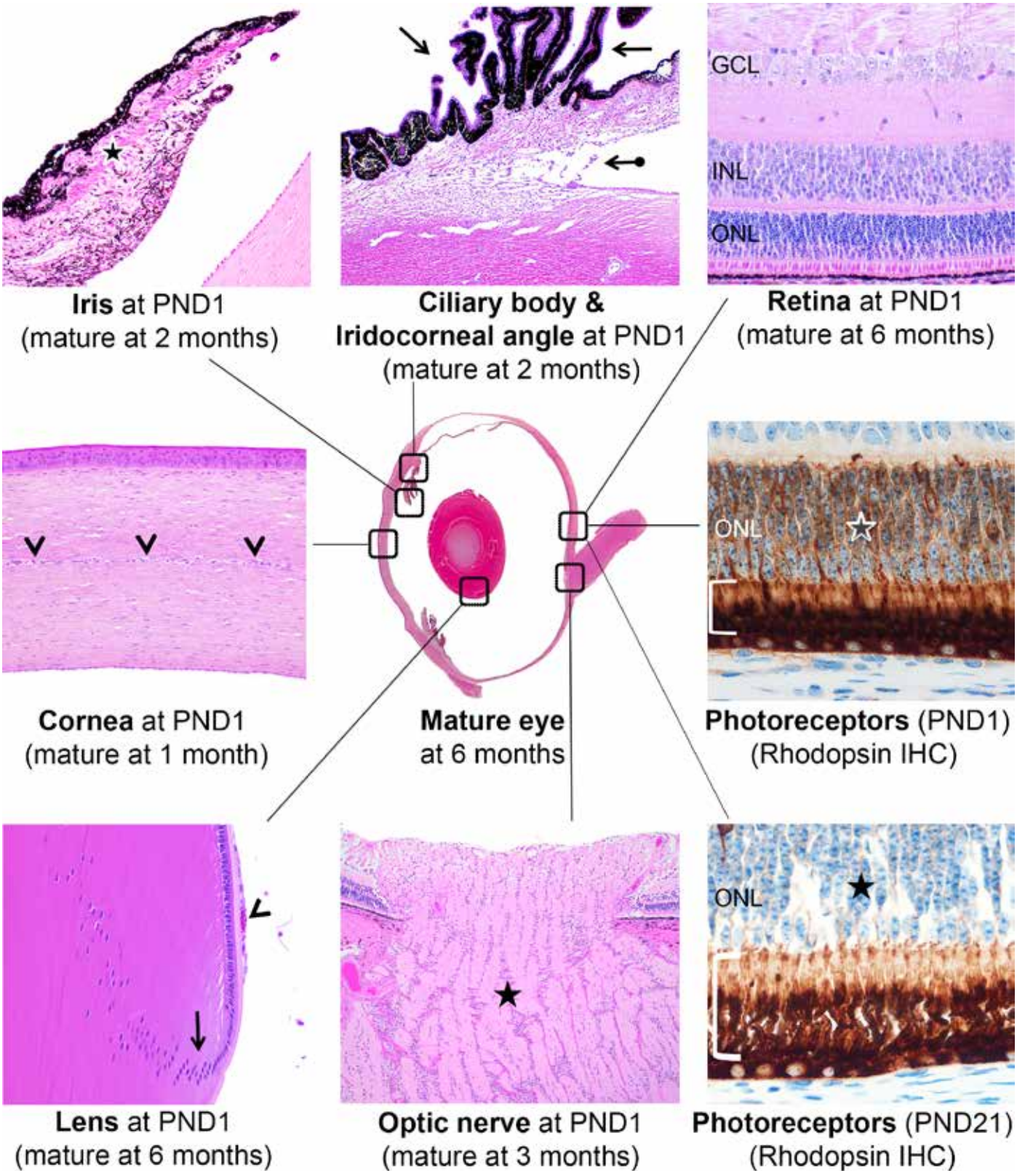


Figure 1

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.

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 Nov;57(6):889-914.
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

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.

Taking care of the pregnant sow and newborn piglets

Practical considerations for the use of Göttingen Minipigs in developmental toxicology, juvenile toxicology, and lactation studies

By Kirsten Rosenmay Jacobsen[1]

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

Göttingen Minipigs are a well-known non-rodent species used for developmental and juvenile toxicity studies (1, 2). To ensure research quality and animal welfare, knowledge about the normal reproductive characteristics of the Göttingen Minipigs, correct handling of the sow before, during, and after farrowing, and the care of the newborn piglets is critical.

Gestation

The gestation period for Göttingen Minipigs lasts for an average of 115 days. Sows that have fewer piglets or are breeding for the first time may experience delayed farrowing. However, Göttingen Minipigs have a low rate of reproductive failure and a high success rate for both pregnancy and farrowing. On average, Göttingen Minipigs give birth to a litter of 8.1 piglets, but this number may vary depending on parity (as shown in Figure 1).

It is possible to synchronize estrus in Göttingen Minipigs, and farrowing can be induced, which allows a group of sows to farrow within a short time frame of typically 24-48 hours. Additionally, the incidence of congenital external and visceral malformations in Göttingen Minipigs is low (3).

Benefits of using Göttingen Minipigs for juvenile studies

- Short gestation length and large litter size compared to non- human primates (115 d, 5-10 piglets)
- Estrus and farrowing can be synchronized
- Well-developed at birth (stand up, walk, suckle, open eyes). Easy to identify weak piglets
- 300-450 g at birth making all dosing routes possible (e.g. PO on PND1) + PK/TK serial sampling and growth measurements at early ages
- Ideal for cross-fostering
- High health status and only needs an iron injection

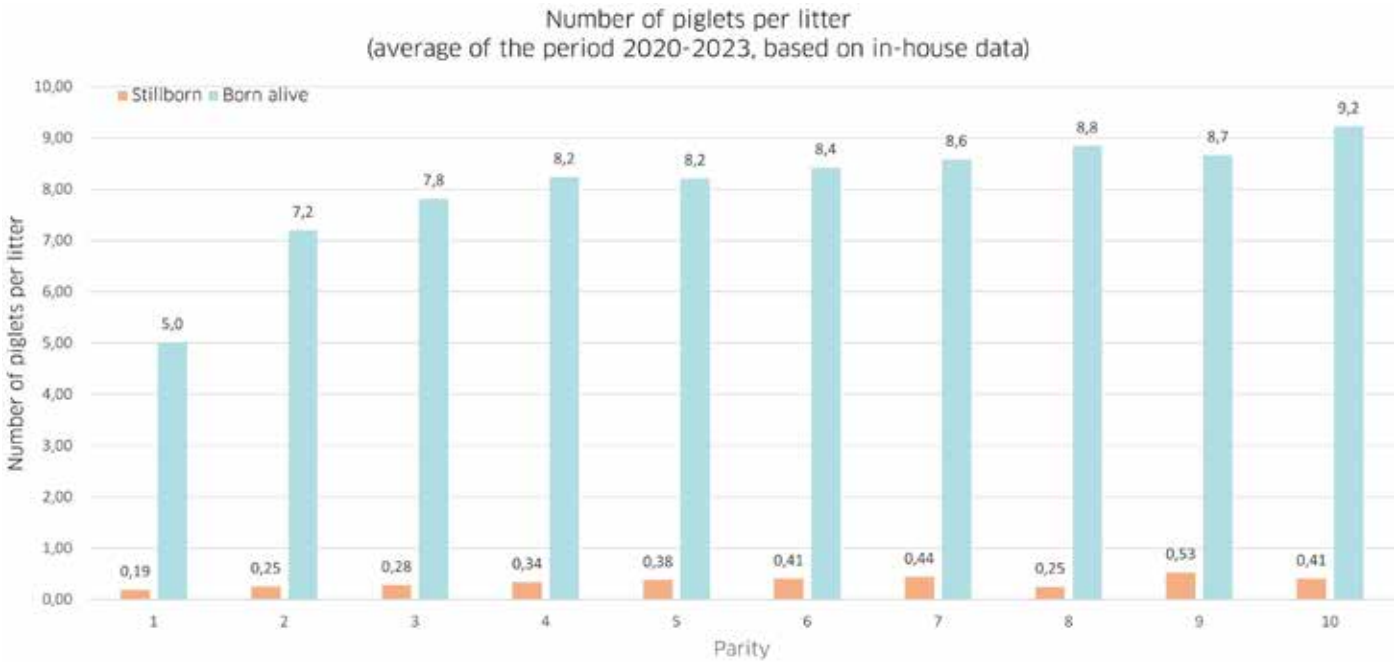


Figure 1
Average number of piglets born alive (in turquoise) and stillborn (in orange) in the period 2020-2023 divided into parity at Ellegaard Göttingen Minipigs, Dalmose, Denmark.

Picture 1
Sow after farrowing. The provision of material for nest building not only improves the wellbeing of the sows, but also gives a good indication of imminent farrowing.

Taking care of the sow

Pregnant sows should be transferred to the farrowing pen 7–10 days prior to the farrowing date. The farrowing pen should be designed to provide the best environment for the sow and the piglets e.g. space and opportunity for the sow to perform nest building, bars to protect the piglets from the sow, and an area with additional heat for the newborn piglets.

Socialization of the pregnant sow, for example through hand-feeding or petting/scratching before and after farrowing creates a calm and trusting environment, even when staff members are interacting with the piglets. Having the sow habituated to touching and manipulation also makes it easier to get a sense of how close to farrowing the sow is. As she nears parturition, her udder will be harder, and her teats will have milk in them.

The temperament of the sow has an impact on the piglets and their reaction to humans, and a calm sow typically will foster calm piglets. However, a certain amount of protective maternal behavior is beneficial for the sow to care for her piglets. Primiparous sows may be more nervous than more experienced breeders and it is important to respect the individual sow's temperament in the socialization and care immediately after farrowing.

It is essential for sows to get the correct amount of feed up to farrowing, so that they have enough energy to farrow, but also to avoid problems with constipation and prolonged farrowing. Sup-optimal energy and dietary conditions may also affect milk production after farrowing. The eating habits and general condition of the sow must be observed closely, and the amount of diet should be decreased or increased accordingly. Sows with problems keeping their weight should be fed multiple small meals daily and treats (e.g. fruit, juice, yogurt) until normal body condition is regained.

The sows must have access to plenty of fresh clean water, either from a water nipple supplying a minimum of 2–4 liters per minute or from a well-attached bowl.

The ambient temperature at sow height should be 18–20°C.

Parturition

Sows usually become more restless a day or two before farrowing and start nest-building behavior. To facilitate this, it is important to provide sufficient bedding material in the pen (as shown in Picture 1).

When a sow lies down in the nesting area and begins to go into labor, it is essential not to disturb her. Sows usually give birth during the night, and they usually don't need any assistance. However, if the sow has been in labor for more than two hours, and no piglets are born, take her out of the pen and walk her for 5-10 minutes in the aisle. It is good if the sow urinates while she is up and walking. The time it takes from the first piglet's birth until the last piglet and the placenta are expelled varies greatly. The sow may eat the placenta which is thus not always observable in the pen. After farrowing, the sow must be checked to confirm she is alert and interested in the piglets. A veterinary examination must be performed if there are any signs of clinical disease, depressed behavior, or refusal to nurture the piglets, and treatment should be initiated accordingly.

Göttingen Minipigs can be milked and used in lactation studies to evaluate the transfer of drugs from the sow to the piglets through the milk. A suitable method for milking is based on positive reinforcement training, where the desired behavior is rewarded with a treat. Training should start weeks prior to farrowing by training the sow to accept being touched and simulated milking. In addition, it is beneficial to train the sows to stand with the front legs on a step board or similar object during the milking process. The board has two main functions. It gives the sow a focus point, and it helps to overcome the anatomical challenge of the teats being close to the ground on postpartum minipigs, thus providing working space for the milking process (Ellegaard Göttingen Minipigs, Internal training protocol for milking Gottingen Minipigs).

Colostrum can be sampled immediately after farrowing without previous training of the sow as the production is high. Colostrum can be frozen without losing its immunological properties (4) and can be used when needed. Colostrum should be heated gently to 37 °C before administration and used immediately after being thawed. Although parity has no effect on the concentrations of immunoglobulins in colostrum (5), we recommend collecting colostrum from multiparous sows, since primiparous sows yield less amount (6). Furthermore, it is important to eliminate potential stress factors (such as milking) for first-time sows.

The newborn piglet

From 6 to 48 hours after birth, piglets require an iron supplement to prevent anemia (7). At this time, each piglet should be marked with their individual animal identification number to ensure proper recordkeeping. Identification can be by a chip, ear notching, or temporary marking with a dye.

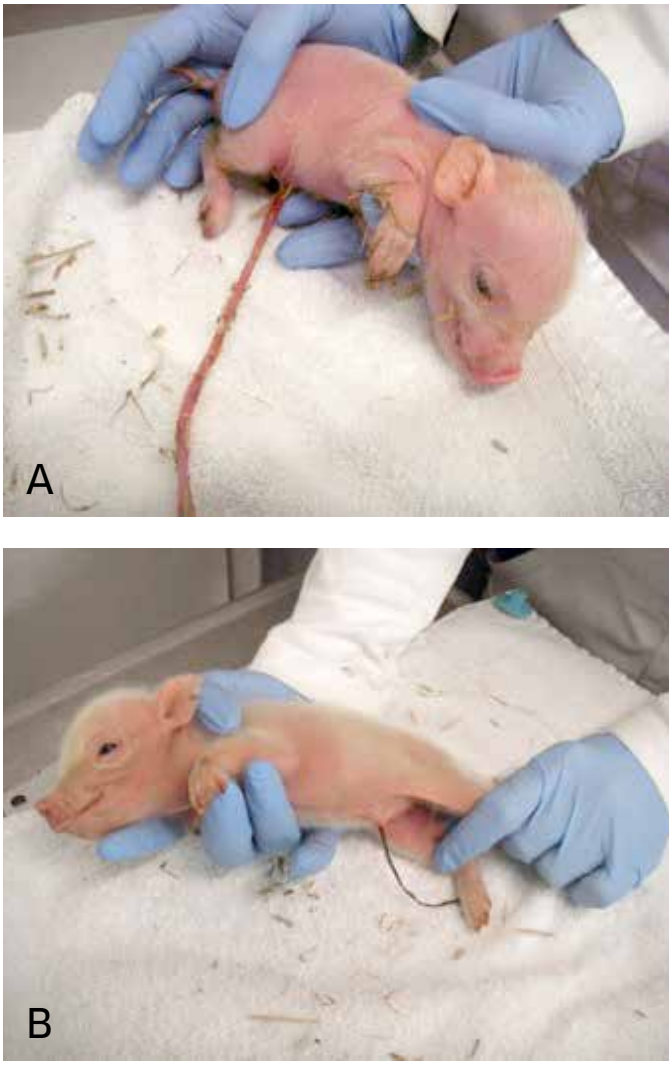
Maternal antibody transfer is entirely via colostrum and not the placenta. Consumption of sufficient amounts of colostrum within the first few hours of life is thus essential for the survival and subsequent well-being of newborn piglets (8, 9). The piglets should be satisfied and alert with a rounded rather than sunken belly – in the latter case, this could indicate insufficient intake of colostrum. To increase the chance of survival for cold and weak-born piglets, colostrum can be administrated orally with a needleless syringe multiple times within the first 24 hours of life. In general, piglets should not be given more than 1 ml colostrum at a time, as larger amounts increase the risk of accidental inhalation and subsequent risk of pneumonia.

In the piglet area, the temperature must be at least 34–37°C for the first week. After that, the temperature in the area should be gradually reduced. If the piglets are cold, it may reduce their ability to absorb antibodies in the gut and they will need extra energy to raise their body temperature and maintain their metabolism. Thus, keeping the piglets warm will increase the survival rate (Picture 2).

Picture 2
If piglets are removed from the sow, they must be kept warm, for example by placing an electric heating blanket or a soft warming cover in the box the piglets are transported in. The time separated from the sow should be as short as possible.

Cross-fostering

Cross-fostering can be done if piglets have to be removed from an aggressive sow, to equalize litter size between sows, and for research purposes. It is crucial that all the piglets get colostrum before cross-fostering takes place, and it is recommended not to remove the piglets until their umbilical cord is dry (see Picture 3). To be certain that all piglets get a functioning teat, cross-fostering allocation must be completed preferably no more than 48 hours after farrowing. The piglets that are moved must be strong enough to handle the move, and it is advisable to remove the largest piglets from the large litter and mix them in a small warm area (34–37°C) with the piglets from the smaller litter that they are allocated to. The mixed piglets should remain together for about 30 minutes to make their scent uniform before introducing them to the sow . To stimulate milk production and facilitate the acceptance of the new piglets, the foster sow can be given ½ ml oxytocin IM in the neck muscle. It is imperative to observe the sow and her behavior for a while to make sure the piglets are accepted.



Picture 3
A: Newborn Göttingen Minipig with a fresh umbilical cord.
B: The umbilical cord is now dry, the belly is rounded as a sign that the piglet has received sufficient colostrum and the piglet is now ready to be placed with a foster sow.

REFERENCES

1. Mikkelsen LF, Van Cruchten S, Makin A: The use of Göttingen Minipigs in juvenile studies. in Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays. Edited by Hock FJ, Gralinski MR, Pugsley MK. Cham, Springer International Publishing; 2022. pp. 1-19.
2. Svendsen O. The minipig in toxicology. Exp Toxicol Pathol. 2006;57:335-339.
3. Ellemann-Laursen S, Marsden E, Peter B, Downes N, Coulby D, Grossi AB. The incidence of congenital malformations and variations in Göttingen minipigs. Reprod Toxicol. 2016;64:162-168.
4. Ramírez-Santana C, Pérez-Cano FJ, Audí C, Castell M, Moretones MG, López-Sabater MC, Castellote C, Franch A. Effects of cooling and freezing storage on the stability of bioactive factors in human colostrum. J Dairy Sci. 2012;95:2319-2325.
5. Segura M, Martínez-Miró S, López MJ, Madrid J, Hernández F. Effect of Parity on Reproductive Performance and Composition of Sow Colostrum during First 24 h Postpartum. Animals (Basel). 2020;10.
6. Nuntapaitoon M, Juthamanee P, Theil PK, Tummaruk P. Impact of sow parity on yield and composition of colostrum and milk in Danish Landrace × Yorkshire crossbred sows. Preventive Veterinary Medicine. 2020;181:105085.
7. National Research Council: Nutrient Requirements of Swine. 11th edition ed, The National Academies Press; 2012.
8. Dividich JL, Esnault T, Lynch B, Hoo-Paris R, Castex C, Peiniau J. Effect of colostral fat level on fat deposition and plasma metabolites in the newborn pig. Journal of Animal Science. 1991.
9. Quesnel H, Farmer C, Devillers N. Colostrum intake: Influence on piglet performance and factors of variation. Livestock Science. 2012;146:105-114.

Challenges of dermal administration in the juvenile Minipig

By Laure Penard, Senior Toxicologist[1]

[1]*Charles River Laboratories, Lyon, France*

Introduction

The Göttingen Minipig has now become widely accepted as a non-rodent species for safety testing, including juvenile safety testing due to its availability and comparability to man. The Minipig has many advantages such as a relatively large litter size, rapid growth rate, rapid achievement of sexual maturity and piglets can be used for laboratory procedures from a very early age, even from postnatal day (PND1) onwards. Due to the relatively recent use of the Minipigs for juvenile studies, only a small number has been conducted and experience with this model is limited. In the work presented here, we set up the dermal administration technique for neonatal Minipigs from as early as PND1.

Method

Three litters of newborn Göttingen Minipigs were allocated to a dermal feasibility study with the aim of starting administration from PND1.

Piglets from the first litter (n=8) were not treated with any compound and were used uniquely for establishing technical procedures and materials. Piglets from the other two litters (n=12 in total) were treated dermally with a gel placebo (volume of 2 mL/kg/day) applied on both flanks, defined with anatomic landmarks (between the scapulae and hipbones). The treated area was occluded for 4 to 6 hours per day.

To evaluate the feasibility of dermal administration, the piglets were observed daily for clinical observations and local tolerance. They were socialized to evaluate the interactions between the mother and piglets and between piglets. The application area and the total body surface were measured between PND1 to 8 weeks of age in order to confirm regulatory compliance1 (application on at least 10% of the body surface).

Several dressings were tested to cover the application area, depending on the weight of piglets. For piglets at birth (body weight <0.5 kg), we used a porous gauze dressing kept in place with a tubular net bandage and surgical tape (Figure 1a). Then, the porous gauze dressing was covered by a jacket of different sizes (normally used for implanted animals): jacket for juvenile animals for piglets >0.5 kg (Figure 1b) and then jacket for adult Minipigs from 4 weeks of age (2-2.5 kg) (Figure 1c).

Figure 1
Dressing for dermal application adapted to the body weight of piglets.
A: for piglets <0.5 kg - B: for piglets >0.5 kg - C: for piglets over 2-2.5 kg.nipigs.

Reprint notice

Originally published in the Ellegaard Göttingen Minipigs' Newsletter #47, Winter edition 2016.

Author's comment:
This work was also the opportunity to study and publish on the specificities of skin morphology in Juvenile Minipigs (see DOI 10.1177/0192623318804520 for the references).

This method was applied and confirmed in non GLP and GLP studies in piglets aged of 6 days.

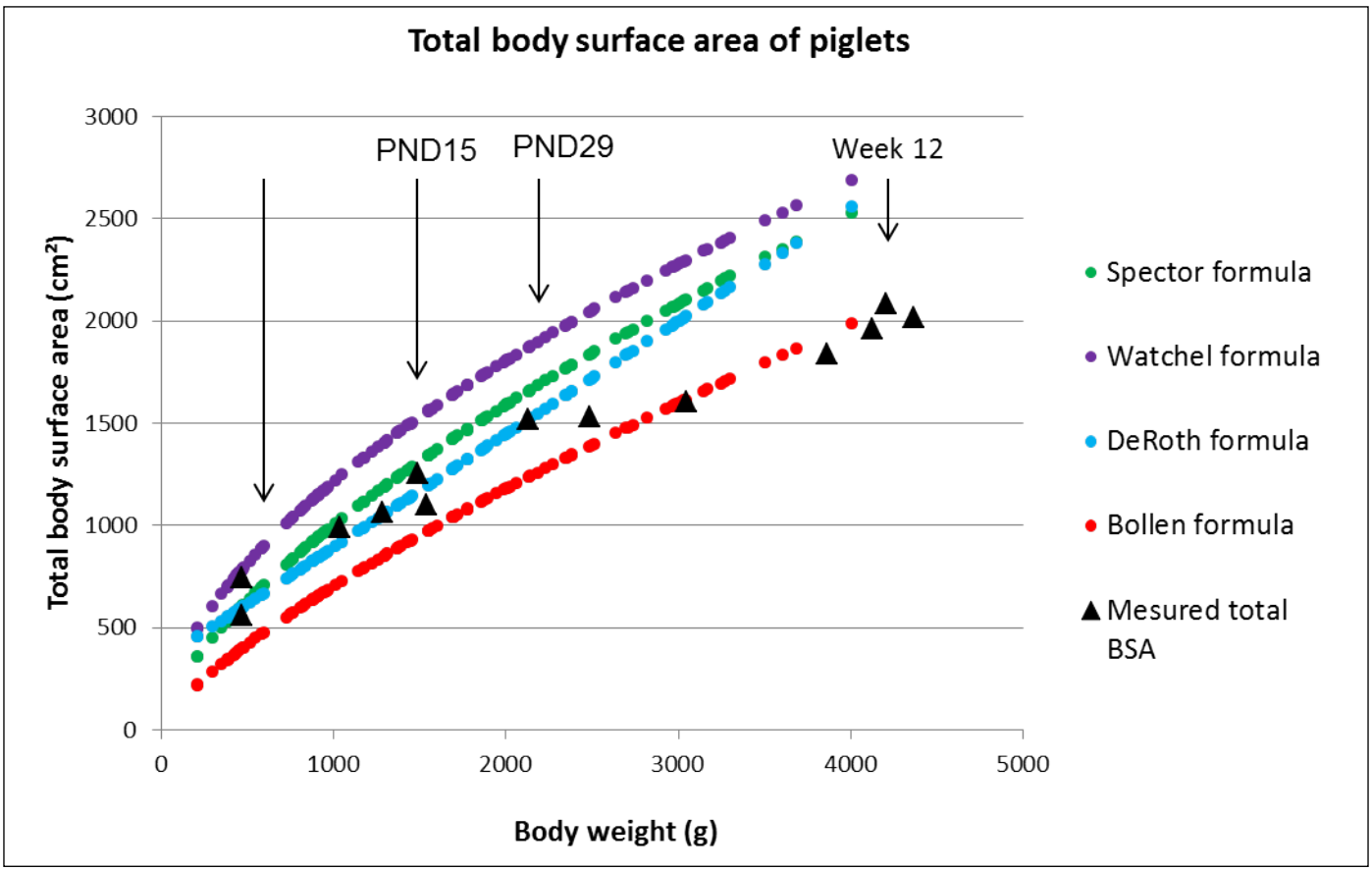


Figure 2
Measured total body surface area (BSA) of piglets compared to usual methods of calculation.

Results

The dressing materials and placebo were well tolerated locally and there was no effect on body weight and tibia length when compared with background data.

We demonstrated that:

- Anatomical landmarks can be used to determine the application area in the juvenile Minipig at least up to 12 weeks of age.
- At least 10% of the total body surface was treated dermally throughout the study despite the rapid piglet growth.
- Our results confirmed that the theoretical total body surface area calculated using the Spector formula2 was similar or slightly superior to the actual total body surface up to 12 weeks of age (4.5 kg).
- For a more accurate estimation of total body surface area, DeRoth formula3 and Bollen formula4 can be used for piglets depending on the body weight (inferior or superior to 2 kg, respectively) (Figure 2).

Conclusion

In conclusion, dermal administration to litters of suckling piglets is feasible from PND1. The dressing materials and placebo were well tolerated locally and had no impact on physical development. We demonstrated that anatomical landmarks (area between the scapulae and hipbones) can be used to determine the application area in the neonatal and juvenile Minipig, at least up to 12 weeks of age (4.5 kg) and that at least 10 % of the total body surface area was treated dermally throughout the study, despite rapid piglet growth. The brief periods during which the newborn piglets were removed from the mother for technical procedures had no impact on their health status so adequate transfer of colostrum was assumed. Our results also confirmed that the theoretical total body surface area, calculated using DeRoth formula for piglets < 2 kg and then Bollen formula for piglets > 2 kg, was similar to the actual total body surface up to 12 weeks of age.

REFERENCES

1 Regulatory guideline requirements (OECD 410 Repeated Dose Dermal Toxicity Study; EPA-Health Effects Test Guidelines-OPPTS 870,3200)
2 Spector formula: BSA (cm^2) = 10 x BW(g)2/3 (Handbook of Biological Data. Philadelphia: W.B. Saunders; 1956)
3 DeRoth formula for pig<2kg:BSA (cm^2) = 337,2 + 0,552 x BW(g) (DeRoth and Bisaillon. Determination of body surface area in neonatal swine. Lab. Anim. Sci. 29(2):249-250.1979) (Swindle. Surgery, anesthesia and experimental techniques in swine. 1st Ed. Iowa State University Press. 1998, p230)
4 Bollen formula: BSA (cm^2) = 700 x BW(kg)0,75 (Bollen and al. The Laboratory Swine, Second Edition. CRC Press. 2010, p87) (Willard-Mack and al. Dermatotoxicology: Safety Evaluation of Topical Products in Minipigs: Study Designs and Practical Considerations. Toxicol. Pathol., 2016)

Recommended review papers on Göttingen Minipigs in juvenile studies

The following selection of papers lists publications on the use of Göttingen Minipigs in juvenile studies. The list is not complete but includes an excerpt of papers, which have contributed to the general understanding and characterisation of Göttingen Minipigs as a large animal model.

Ellegaard Göttingen Minipigs strives at collecting all available knowledge and relevant publications about Göttingen Minipigs in biomedical research. If you are looking for something specific, or references to a different topic, please contact ellegaard@minipigs.dk.

Juvenile and neonatal studies

Lenz JH, Henkel KO, Schmidt W, et al.
Ossification of the frontal sinus in juvenile minipigs following water jet application
Journal of Cranio-Maxillo-Facial Surgery
Published: Mar 2006
DOI: 10.1016/j.jcms.2005.09.002

Haagensen AMJ, Grand N, Klastrup S, et al.
Spatial discrimination and visual discrimination: two methods evaluating learning and memory in juvenile Göttingen minipigs
Behavioural Pharmacology
Published: Jun 2013
DOI: 10.1097/FBP.0b013e32836104fd

Lorenzen E, Kudirkiene E, Gutman N, et al.
The vaginal microbiome is stable in prepubertal and sexually mature Ellegaard Göttingen Minipigs throughout an estrous cycle
Veterinary Research
Published: Oct 2015
DOI: 10.1186/s13567-015-0274-0

Peer EV, Downes N, Casteleyn C, et al.
Organ data from the developing Göttingen minipig: first steps towards a juvenile PBPK model
Journal of Pharmacokinetics and Pharmacodynamics
Published: Apr 2016
DOI: 10.1007/s10928-015-9463-8

Feyen B, Penard L, Heerden MV, et al.
"All pigs are equal" Does the background data from juvenile Göttingen minipigs support this?
Reproductive Toxicology
Published: Sep 2016
DOI: 10.1016/j.reprotox.2016.04.019

Peer EV, Jacobs F, Snoeys J, et al.
***In vitro* Phase I- and Phase II-Drug Metabolism in The Liver of Juvenile and Adult Göttingen Minipigs**
Pharmaceutical Research
Published: Apr 2017
DOI: 10.1007/s11095-017-2101-y

Siefert J, Hillebrandt KH, Kluge M, et al.
Computed tomography-based survey of the vascular anatomy of the juvenile Göttingen minipig
Laboratory Animals
Published: Aug 2017
DOI: 10.1177/0023677216680238

Gauthier BE, Penard L, Bordier NF, et al.
Specificities of the Skin Morphology in Juvenile Minipigs
Toxicologic Pathology
Published: Oct 2018
DOI: 10.1177/0192623318804520

Siefert J, Hillebrandt KH, Moosburner S, et al.
Hepatocyte Transplantation to the Liver via the Splenic Artery in a Juvenile Large Animal Model
Cell Transplantation
Published: Dec 2019
DOI: 10.1177/0963689719885091

Smits A, Annaert P, Cruchten SV, Allegaert K
A Physiology-Based Pharmacokinetic Framework to Support Drug Development and Dose Precision During Therapeutic Hypothermia in Neonates
Frontiers in Pharmacology
Published: May 2020
DOI: 10.3389/fphar.2020.00587

Vrolyk V, Desmarais MJ, Lambert D, et al.
Neonatal and Juvenile Ocular Development in Göttingen Minipigs and Domestic Pigs: A Histomorphological and Immunohistochemical Study
Veterinary Pathology
Published: Nov 2020
DOI: 10.1177/0300985820954551

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
Published: Nov 2020
DOI: 10.3390/metabo10110456

Allais L, Brisebard E, Ravas N, et al.
Skin immune cell characterization in juvenile and adult Göttingen Minipigs
Regulatory Toxicology and Pharmacology
Published: Mar 2021
DOI: 10.1016/j.yrtph.2021.104861

Valenzuela A, Tardiveau C, Ayuso M, et al.
Safety Testing of an Antisense Oligonucleotide Intended for Pediatric Indications in the Juvenile Göttingen Minipig, including an Evaluation of the Ontogeny of Key Nucleases
Pharmaceutics
Published: Sep 2021
DOI: 10.3390/pharmaceutics13091442

Allais L, Perbet A, Condevaux F, et al.
Immunosafety evaluation in Juvenile Göttingen Minipigs
Journal of Immunotoxicology
Published: Dec 2022
DOI: 10.1080/1547691X.2022.2088904

Stroe MS, Bockstal LV, Valenzuela A, et al.
Development of a neonatal Göttingen Minipig model for dose precision in perinatal asphyxia: technical opportunities, challenges, and potential further steps
Frontiers in Pediatrics
Published: May 2023
DOI: 10.3389/fped.2023.1163100

Leys K, Stroe MS, Annaert P, et al.
Pharmacokinetics during therapeutic hypothermia in neonates: from pathophysiology to translational knowledge and physiologically-based pharmacokinetic (PBPK) modeling
Expert Opinion on Drug Metabolism & Toxicology
Published: Jul-Dec 2023
DOI: 10.1080/17425255.2023.2237412

Sun J, Chong J, Zhang J, Ge L
Preterm pigs for preterm birth research: reasonably feasible
Frontiers in Physiology
Published: Jul 2023
DOI: 10.3389/fphys.2023.1189422

NEWS FROM

Ellegaard Göttingen Minipigs A/S

Ellegaard Göttingen Minipigs A/S supply Göttingen Minipigs for biomedical research around the world. From our AAALAC accredited facility we breeed Göttingen Minipigs to enable development of safer and more effective medicines, all based on our core values: Animal welfare, quality, respect, and collaboration.

Appointments and anniversaries

17 December 2023 Lars Nielsen celebrated his 5th anniversary with Ellegaard Göttingen Minipigs.

Lars is one of our experienced Animal Caretakers in the breeding barriers, who ensures the well-being of our Göttingen Minipigs every day.

1 January 2024 Kamilla Flemming Hansen celebrated her 5th anniversary with Ellegaard Göttingen Minipigs as well.

Kamilla is our Laboratory Technician and a skilled surgeon, often engaged in customer projects.

1 February 2024 we also celebrated the 5th anniversary of Mette Damgaard Johansen.

Mette is part of our Order Management Department and takes good care of our customers and plans safe travelling schedules for our Göttingen Minipigs.

Where to meet us in 2024

CONFERENCE	DATE	LOCATION
SOT & ToxExpo	10-14 Mar	Salt Lake City, UT, USA
IAT	12-15 Mar	Scotland
Tierschutz-Tagung Travemünde	23-24 Apr	Lübeck, Germany
Scand-LAS	21-24 May	Tampere, Finland
Minipig Research Forum (MRF)	22-24 May	Amsterdam, The Netherlands
AFSTAL	12-17 Jun	Lille, France
SBR	14-18 Jun	Madison, WI, USA
EUROTOX	8-11 Sep	Copenhagen, Denmark
GV-Solas & IGTP	11-13 Sep	Würzburg, Germany
SPS	22-25 Sep	San Diego, CA, USA
ACT	17-20 Nov	Austin, TX, USA

Spotlights

Follow the barrier-bred Göttingen Micropigs

Meet No. 500553, one of the first barrier-bred Göttingen Micropigs. In the photo, he stands next to a Göttingen Minipig. They were born on the same day and in the photo they are both 2.5 months old. At this age Göttingen Minipigs weigh around 6 kgs, but No. 500553 only weighs 2,9 kgs.

Through genetic alteration, the inactivation of the Growth Hormone Receptor (GHR) gene has resulted in the development of a novel porcine strain: Göttingen Micropigs. Through targeted genetic intervention, researchers have achieved growth retardation, resulting in the establishment of a distinct breed characterized by miniature proportions.

Follow No. 500553 on LinkedIn as we share additional information and data about his growth and development over the coming months and get a glimpse of the potential of the new Göttingen Micropigs strain.

linkedin.com/company/ellegaard-gottingen-minipigs

Health Monitoring Report: December 2023

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

Principal Laboratory Animal Veterinarian at Ellegaard Göttingen Minipigs, Maja Ramløse, who is responsible for reviewing the overall health monitoring plan, collecting, accumulating, and reporting the results, says: "We monitor the health of our colonies twice a year for a wide range of pathogens. In May/June we screen for selected agents, and in November/December we perform an extended analysis. For the latest report, 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, Cryptosporidium. Repeated re-testing suggest a low prevalence and our overall assessment is that this finding is of limited importance. For your convenience additional information on this finding is reported. As usual, an exclusion list alongside our health monitoring report is reported, to give a quick overview of which agents prompt either authority notification or further diagnostic or corrective action."

Download the full report, additional information on findings, and an exclusion list from minipigs.dk/about-gottingen-minipigs/health-status.

Study case: Placental and milk drug transfer in a juvenile study in Göttingen Minipigs

Laboratory Animal Research Veterinarian at Ellegaard Göttingen Minipigs, Susi Søgaard, shares her insights:

Göttingen Minipigs offer a unique advantage in research due to their high compatibility with humans in anatomy, physiology, and biochemistry. This species was therefore selected in a study that aimed to investigate if test items could cross the placenta allowing the piglets to be exposed to and born with a desired concentration of the test item. The Göttingen Minipigs have high reproductive capabilities securing many healthy and relatively large offsprings pr. mother which resulted in large study groups, and the possibility to cross-foster making them inter- and intralitter control animals.

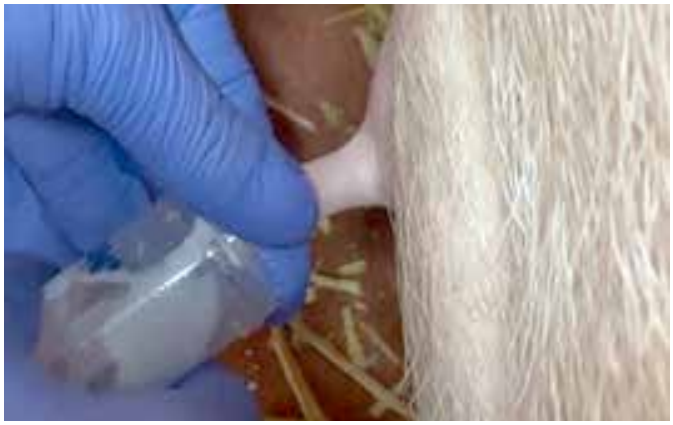
The sows were divided into three groups receiving a daily doses of different test items from five (5) weeks before parturition until weaning four (4) weeks after parturition. Blood and milk were collected from the sows. Blood and tissue (at necropsy) were collected from the piglets.

In this study, cross-fostering was used to randomize the treatment groups and investigate the exposure in utero (transplacental transfer) and during lactation (DRUG concentration in milk) by comparing PK profiles from siblings and cross-fostered littermates, where the sows were treated with different test items.

Sows can even when they are pregnant. In this study the animals were sedated with a midazolam/ketamine mix and an ear vein catheter was inserted five (5) weeks before expected farrowing. This allowed for easier and more frequent blood sampling (weekly, before and after parturition) with improved animal welfare and a safer work environment.

Blood was sampled from the piglets weekly over a course of four (4) weeks starting after birth:

- In the first two weeks of age, starting from 24 h after birth, 300 µL from v. saphena by micro sampling

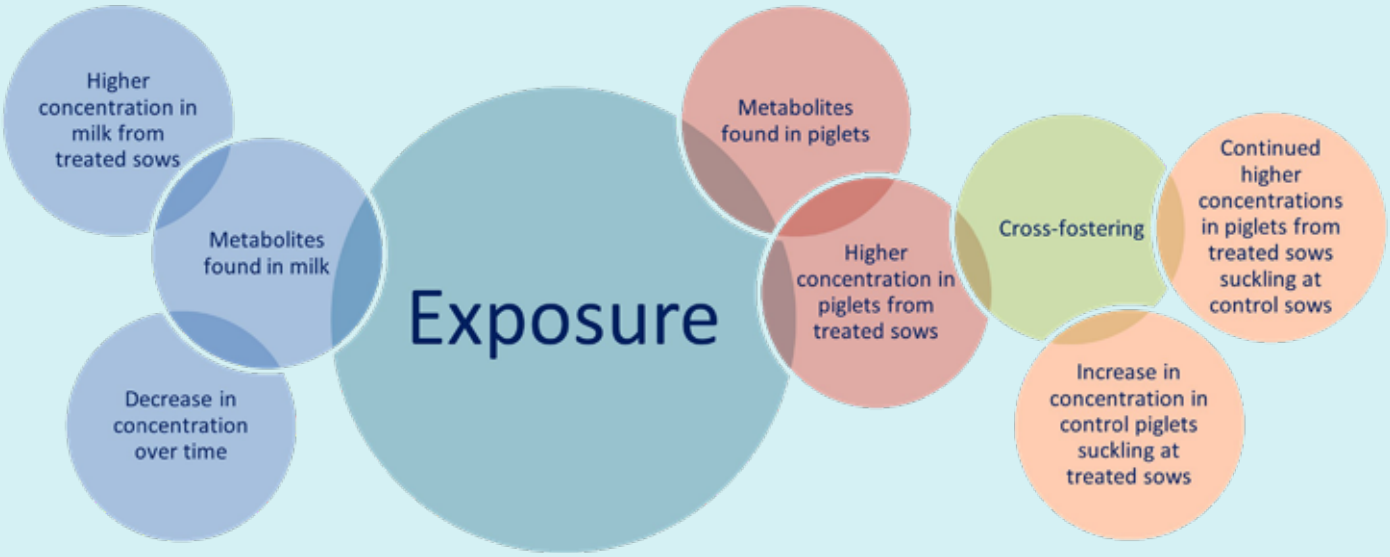


- From 2 weeks of age onwards 500 µL from v. jugularis by vacutainer
- Exposure was confirmed in all piglets that received the test item either in utero or after parturition through the milk

Milk was collected weekly from the sows to measure test item concentration in milk and thereby estimate the possible maximum drug transfer to the piglets. This was done while the sow was nursing the piglets, by removing a piglet and milk the free teat. Metabolites was found in the milk of treated sows.

Figure below: Visualization of the measured exposure in the milk (sows) and the piglets (blood).

Test item metabolites were measured in all milk samples and a decrease in concentration was seen over the nursing period (blue circles). 24 h after birth a higher concentration was measured in piglets born from treated sows (red circles). After cross-fostering two interesting observations were made. Piglets that had received the transplacental transfer (born from treated sows) continued to have higher concentrations of the test item, even when they were nursed by a non-treated sow, and therefore did not receive the test items through the milk. Control piglets born from non-treated sows (with a low initial concentration) had an increase in concentration when they were nursed by treated sows (received the test item through the milk) (orange circles).



GÖTTINGEN MINIPIGS ACADEMY

Who can join?

The academy facilitates seminars and workshops on various topics concerning Göttingen Minipigs, designed for those working within the life sciences industry. Courses target a diverse audience and are carefully selected to accommodate the needs of:

- **Animal Technicians**
- **Animal Caretakers**
- **Laboratory Technicians**
- **Veterinarians**
- **Researchers**
- **Scientists**
- **Study Directors**
- **Sponsor Monitors**
- **Surgical Staff**

Yet, anyone with an interest in the welfare, management, and use of Göttingen Minipigs in biomedical research can benefit from the courses.

The instructors/lecturers

All courses are developed and conducted by experienced professionals and experts within their respective fields, covering various aspects of working with Göttingen Minipigs, incl. their biology and behaviour, husbandry, veterinary management and welfare, hands-on practical exercises, and animal models. From 2024 we also conduct courses on ergonomics, animal training, disease models, and specific study types.

Customised and personal courses

All courses can be customised or conducted as personal sessions. For enquires or questions about course content or the Academy in general, please contact academy@minipigs.dk.

Former course participants

Time well spent - a good mix of theory and hands-on training for new as well as experienced employees, boosting the qualifications of our minipig facility team

Helene Kringel
Principal Scientist, Pharmacology Researach | Gubra

Perfect course day with a good overview of the most common dosing methods followed by practical training. Very rewarding!

Hanna Lindgren
Animal Technician | AstraZeneca

Being able to test new methods for fixing and dosing minipigs for one day gave our team a good start to develop new knowledge and skills that we use daily in our work.

Emma Kvarnström
Veterinary Nurse | AstraZeneca

I attended one of the first Göttingen Minipigs Academy workshops, and it was a great experience and very instructive. I have been working with Göttingen Minipigs for more than 10 years, and one thing I know, is that there is always new things to learn.

The workshop was a good combination of theory and hands on work – and one of the best things was for sure that the workshop was adapted to us course participants and conveyed by very skilled personnel from Ellegaard.

I will recommend everyone working with the minipig to participate in one of the workshops – there is always more to learn, and the Göttingen Minipigs Academy is the place to learn it.

Trine Starostka
DVM and Senior Scientist | Minerva Imaging

Scheduled courses

Göttingen Minipigs & Veterinary Management
Introductory Course

Date 4 Mar and 7 Oct, 3:00-5:00 pm
Location Online
Price Free
Participants Veterinarians, Animal Caretakers, Laboratory Technicians, Students

Click or scan for course description

Ergonomics & Well-Being in the Animal Facility

Date 2 Sep, 9:00 am - 2:00 pm
Location Dalmose, Denmark
Price € 500
Participants Veterinarians, Animal Caretakers, Laboratory Technicians

Click or scan for course description

Anaesthesia & Analgesia

Date 8 Apr, 1:00-4:00 pm
Location Dalmose, Denmark
Price € 340
Participants Laboratory Technicians, Veterinarians, Animal Caretakers, Surgical Staff

Click or scan for course description

How to Train Göttingen Minipigs

Date 18 Nov, 9:00 am - 3:00 pm
Location Dalmose, Denmark
Price € 1,300
Participants Veterinarians, Animal Caretakers, Laboratory Technicians

Click or scan for course description

Handling & Dosing

Date 10 Jun, 9:00 am -3:30 pm
Location Dalmose, Denmark
Price € 1,300
Participants Veterinarians, Animal Caretakers, Laboratory Technicians

Click or scan for course description

Diet-Induced Disease Models

Date 5 Dec, 3:00-4:00 pm
Location Web Academy / Online
Price Free
Participants Pharma, Scientists, Veterinarians, Study Directors, Sponsor Monitors

Click or scan for course description

Course: Handling & Dosing

Course: Handling & Dosing

On-demand

Veterinary Management, Welfare & Culture of Care
Advanced Learners

Duration 9:00 am - 3:00 pm
Location Dalmose, Denmark
Price € 500
Participants Veterinarians, Animal Caretakers, Laboratory Technicians

Click or scan for course description

Vascular Access

Duration 9:00 am - 3:00 pm
Location Dalmose, Denmark
Price € 1,300
Participants Laboratory Technicians, Veterinarians, Animal Caretakers, Scientists

Click or scan for course description

Course: Vascular Access

Course: Vascular Access

The 16th Minipig Research Forum

Join the 16th Minipig Research Forum - a unique opportunity for minipig users to meet, discuss and share knowledge and experiences within all areas of minipig use related to biomedical research. Take part in this 3-day conference packed with scientific lectures, poster presentations and the opportunity of networking with minipig users from all over the world.

SCIENTIFIC SESSIONS

- **Keynote presentation:**
  Exploring translational immunology, SLA genetics and regulatory frameworks in xenotransplantation
- **Application of disease and efficacy models in Göttingen Minipigs**
- **Göttingen Minipigs DMPK data in species selection and translatability**
- **Göttingen Minipigs in safety pharmacology**
  (Cardiovascular, CNS, and respiratory)
- **The use of imaging applications in Göttingen Minipigs in drug and medical devices research**
  (in vivo, metabolism, and histology)

Each session contains presentations by speakers sharing the latest data and knowledge within their specialist areas. The full scientific program is attached and also available at minipigresearchforum.org.

BREAKOUT SESSIONS

Attendees can choose between two different breakout sessions, one of which will contain a continuing education topic. The topics appear from the full scientific program.

PRACTICALITIES

Starts at	**22 May 2024** (Wednesday) 15:00 hrs CEST (Registration desk opens at 14:00 hrs CEST)
Ends at	**24 May 2024** (Friday) 12:00 hrs CEST
Registration fee	**€495** (Early Bird before 1 March 2024) Later registration fee: €595 The registration fee covers keynote presentation, four scientific sessions, one breakout session of choice, full catering, dinners and networking.
Register	To register, log in to your MRF account and follow the link to the registration page: minipigresearchforum.org
Venue	NN Amsterdam Schiphol Hotel Kruisweg 495 2132 NA Hoofddorp The Netherlands
Accommodation	Accommodation is available at the venue hotel at a special conference rate of €144 per night incl. breakfast when booking via this link. The link is also available via your MRF login. ! ***Please make your room reservations no later than 20 March 2024 to ensure availability.***

POSTER VIEWING SESSION

The very popular poster viewing session is as always part of the program. Posters are accepted either with technical (e.g., tips and tricks) or scientific content (must contain data). We also accept posters from companies supplying material relevant for research and testing using minipigs, e.g. assays kits. Please note that commercial posters without data and/or technical information will be declined.

Read the poster guidelines at minipigresearchforum.org with format requirements and deadlines for submission. **The deadline for submission of poster abstracts is 29 April 2024.** As a tradition, the best poster will be elected during the MRF meeting by the MRF Steering Committee.

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 www.minipigresearchforum.org

Programme 2024

KEYNOTE PRESENTATION

Exploring translational immunology, SLA genetics and regulatory frameworks in xenotransplantation	Sabine Hammer, University of Veterinary Medicine, Vienna, Austria

POSTER SESSION

Poster introduction by authors, incl. poster presentations by the MRF knowledge sharing groups: 1) biomarker group update; 2) introducing the pain-in-research group	Deadline for submission is 29 April 2024 View Poster Guidelines on minipigresearchforum.org

SESSION 1 | APPLICATION OF DISEASE AND EFFICACY MODELS IN GÖTTINGEN MINIPIGS

Pig models for Cystic fibrosis and the Usher syndrome in mechanistic and therapeutic studies	Nikolai Klymiuk, Technical University Munich, Germany
Early life glycemic nutrition and later life metabolic health in mildly obese Göttingen Minipigs	Sietse-Jan Koopmans, University of Wageningen, The Netherlands
Functional and morphological renal changes in a Göttingen Minipigs model of obesity-related and diabetic nephropathy	Henrik Duelund Pedersen, Novo Nordisk, Denmark

SESSION 2 | GÖTTINGEN MINIPIGS DMPK DATA IN SPECIES SELECTION AND TRANSLATABILITY

Considerations for choosing the Minipig as a species for DMPK	John Kendrick, LabCorp Harrogate, United Kingdom
In vitro drug metabolism in Göttingen Minipigs: translation to man	Steven van Cruchten, University of Antwerp, Belgium
Minipig Scaling Factors for in vitro – in vivo clearance extrapolation (IVIVE)	Jennie Roberts, AstraZeneca, United Kingdom

SESSION 3 | GÖTTINGEN MINIPIGS IN SAFETY PHARMACOLOGY (CARDIOVASCULAR, CNS, AND RESPIRATORY)

Göttingen Minipigs in the safety pharmacology core battery, is it similar to dogs and cynomolgus monkeys?	Andrea Greiter-Wilke, F. Hoffmann-La Roche, Switzerland

BREAKOUT SESSIONS

Different techniques of collecting ECG data and use of infusion systems	Short introductory presentations by Maria Korsgaard & Pernille Olesen, Scantox, Denmark
Potential and limitations of Humanized Göttingen Minipigs in development of new antibody therapies	Interactive discussion among session participants facilitated by industry specialists

SESSION 4 | THE USE OF IMAGING APPLICATIONS IN GÖTTINGEN MINIPIGS IN DRUG AND MEDICAL DEVICES RESEARCH (IN VIVO, METABOLISM AND HISTOLOGY)

MRI-guided intraparenchymal injection in the Göttingen Minipig brain using the ClearPoint navigation system	Gael Quesseveur, CRL Lyon, France
Brain functional imaging in the (mini)pig model for nutritional and behavioural neuroscience research	David Val-Laillet, NuMeCan, INRAE, INSERM, Univ Rennes, France
Cases of Combined Special Imaging with Histology from Medical Device Minipig Studies	Nils Warfving, Anapath, Switzerland
GLP-1r PET quantification and image guided surgical targeting of GLP-1r in diabetic minipigs	Charles-Henri Malbert, Aniscan, INRAE & University of Adelaide, France
Molecular imaging in cardiac research - what fits your purpose?	Mette Flethøj Madsen, Minerva Imaging, Denmark

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.

Vallone F, Dushpanova A, Leali M, et al.
Left cardiac vagotomy rapidly reduces contralateral cardiac vagal electrical activity in anesthetized Göttingen minipigs
International Journal of Cardiology | 2023 Sep 7 (E-pub)
DOI: 10.1016/j.ijcard.2023.131349

Juul N, Ajalloueian F, Willacy O, et al.
Advancing autologous urothelial micrografting and composite tubular grafts for future single-staged urogenital reconstructions
Scientific Reports | 2023 Sep 20
DOI: 10.1038/s41598-023-42092-3

Sams A, Haanes KA, Holm A, et al.
Heterogeneous vasomotor responses in segments from Göttingen Minipigs coronary, cerebral, and mesenteric artery: A comparative study
Vascular Pharmacology | 2023 Sep 18 (E-pub)
DOI: 10.1016/j.vph.2023.107231

Tora MS, Neill SG, Lakhina Y, et al.
Tumor microenvironment in a minipig model of spinal cord glioma
Journal of Translational Medicine | 2023 Sep 27
DOI: 10.1186/s12967-023-04531-7

Scherthan H, Geiger B, Ridinger D, et al.
Nano-Architecture of Persistent Focal DNA Damage Regions in the Minipig Epidermis Weeks after Acute γ-Irradiation
Biomolecules | 2023 Oct 13
DOI: 10.3390/biom13101518

Chakraborty N, Holmes-Hampton GP, Gautam A, et al.
Early to sustained impacts of lethal radiation on circulating miRNAs in a minipig model
Scientific Reports | 2023 Oct 28
DOI: 10.1038/s41598-023-45250-9

Strauss I, Agnesi F, Zinno C, et al.
Neural Stimulation Hardware for the Selective Intrafascicular Modulation of the Vagus Nerve
IEEE Transactions on Neural Systems and Rehabilitation Engineering | 2023 Nov 14 (E-pub)
DOI: 10.1109/TNSRE.2023.3329735

Stavropoulos A, Bellon B, Pipenger B, Andersen OZ
Two- and three-piece implants to boost data generation in preclinical in vivo research—A short technical report
Clinical and Experimental Dental Research | 2023 Oct 31 (E-pub)
DOI: 10.1002/cre2.805

Zhang E, Shi Y, Han X, et al.
An injectable and biodegradable zwitterionic gel for extending the longevity and performance of insulin infusion catheters
Nature Biomedical Engineering | 2023 Oct 26 (E-pub)
DOI: 10.1038/s41551-023-01108-z

Kirkeby A, Nelander J, Hoban DB, et al.
Preclinical quality, safety, and efficacy of a human embryonic stem cell-derived product for the treatment of Parkinson's disease, STEM-PD
Cell Stem Cell | 2023 Oct 5
DOI: 10.1016/j.stem.2023.08.014

Dekant R, Bertermann R, Serban J, et al.
Species-differences in the in vitro biotransformation of trifluoroethene (HFO-1123)
Archives of Toxicology | 2023 Dec 6
DOI: 10.1007/s00204-023-03640-y

Starch-Jensen T, Schou S, Terheyden H, et al.
Bone regeneration after maxillary sinus floor augmentation with different ratios of autogenous bone and deproteinized bovine bone mineral an in vivo experimental study
Clinical Oral Implants Research | 2023 Sep 27 (E-pub)
DOI: 10.1111/clr.14186

Böhnke N, Indrevoll B, Hammer S, et al.
Mono- and multimeric PSMA-targeting small molecule-thorium-227 conjugates for optimized efficacy and biodistribution in preclinical models
European Journal of Nuclear Medicine and Molecular Imaging | 2023 Oct 26 (E-pub)
DOI: 10.1007/s00259-023-06474-z

Svetlove A, Ritter CO, Dullin C, et al.
Evaluation of MR-safe bioptomes for MR-guided endomyocardial biopsy in minipigs: a potential radiation-free clinical approach
European Radiology Experimental | 2023 Dec 5
DOI: 10.1186/s41747-023-00391-4

Ng F, Nicoulin V, Peloso C, et al.
***In Vitro* and *In Vivo* Hydrolytic Degradation Behaviors of a Drug-Delivery System Based on the Blend of PEG and PLA Copolymers**
ACS Applied Materials & Interfaces | 2023 Nov 27 (E-pub)
DOI: 10.1021/acsami.2c13141

Bredum SK, Strathe AV, Jacobsen J, et al.
Quantifying energy expenditure in Göttingen Minipigs with the ^{13}C-bicarbonate method under basal and drug-treated conditions
Clinical Nutrition ESPEN | 2023 Nov 10 (E-pub)
DOI: 10.1016/j.clnesp.2023.10.041

Eddy EP, Shet MS, Cataldo M, et al.
Evaluation of dermal toxicity and toxicokinetics of povidone-iodine in Göttingen minipigs
Toxicology and Applied Pharmacology | 2023 Dec 5 (E-pub)
DOI: 10.1016/j.taap.2023.116783

Iacono D, Murphy EK, Stimpson CD, et al.
Low-dose brain radiation: lowering hyperphosphorylated-tau without increasing DNA damage or oncogenic activation
Scientific Reports | 2023 Nov 30
DOI: 10.1038/s41598-023-48146-w

Orciani C, Ballesteros C, Troncy E, et al.
The Spontaneous Incidence of Neurological Clinical Signs in Preclinical Species Using Cage-side Observations or High-definition Video Monitoring: A Retrospective Analysis
International Journal of Toxicology | 2023 Dec 8 (E-pub)
DOI: 10.1177/10915818231218984

Eickelmann C, Lieder HR, Sturek M, et al.
Differences in vasomotor function of mesenteric arteries between Ossabaw minipigs with predisposition to metabolic syndrome and Göttingen minipigs
American Journal of Physiology, Heart and Circulatory Physiology | 2023 Dec 22 (E-pub)
DOI: 10.1152/ajpheart.00719.2023

Wagner J, Luck S, Loger K, et al.
Bone regeneration in critical-size defects of the mandible using biomechanically adapted CAD/CAM hybrid scaffolds: An in vivo study in miniature pigs
Journal of Cranio-Maxillo-Facial Surgery | 2023 Nov 29 (E-pub)
DOI: 10.1016/j.jcms.2023.11.007

Wang LH, Marfil-Garza BA, Ernst AU, et al.
Inflammation-induced subcutaneous neovascularization for the long-term survival of encapsulated islets without immunosuppression
Nature Biomedical Engineering | 2023 Dec 5 (E-pub)
DOI: 10.1038/s41551-023-01145-8

Alogna A, Berboth L, Faragli A, et al.
Lung-to-Heart Nano-in-Micro Peptide Promotes Cardiac Recovery in a Pig Model of Chronic Heart Failure
Journal of the American College of Cardiology | 2024 Jan 2
DOI: 10.1016/j.jacc.2023.10.029

Debes KP, Lyhne MK, Hinz K, et al.
Decreased Heart Rate After Dapagliflozin Treatment In Gottingen Minipigs With Chronic Myocardial Infarction
Journal of Cardiac Failure | Jan 2024
DOI: 10.1016/j.cardfail.2023.10.357

Fenwick AJ, Jani VP, Foster DB, et al.
Common Heart Failure With Preserved Ejection Fraction Animal Models Yield Disparate Myofibril Mechanics
Journal of the American Heart Association | 2024 Jan 16
DOI: 10.1161/JAHA.123.032037

Östman M, Försth P, Hedenqvist P, et al.
Novel Calcium Phosphate Promotes Interbody Bony Fusion in a Porcine Anterior Cervical Discectomy and Fusion Model
Spine | 2024 Jan 12
DOI: 10.1097/BRS.0000000000004916

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
New Drug Development Cluster
593-8, Dongtangiheung-ro,
Hwaseong-si, Gyeonggi-do,
Korea 18469
Tel.: +82 31 888 9369
Fax: +82 31 888 9368
ljhong@woojungbio.kr