No. 68 | Spring 2024

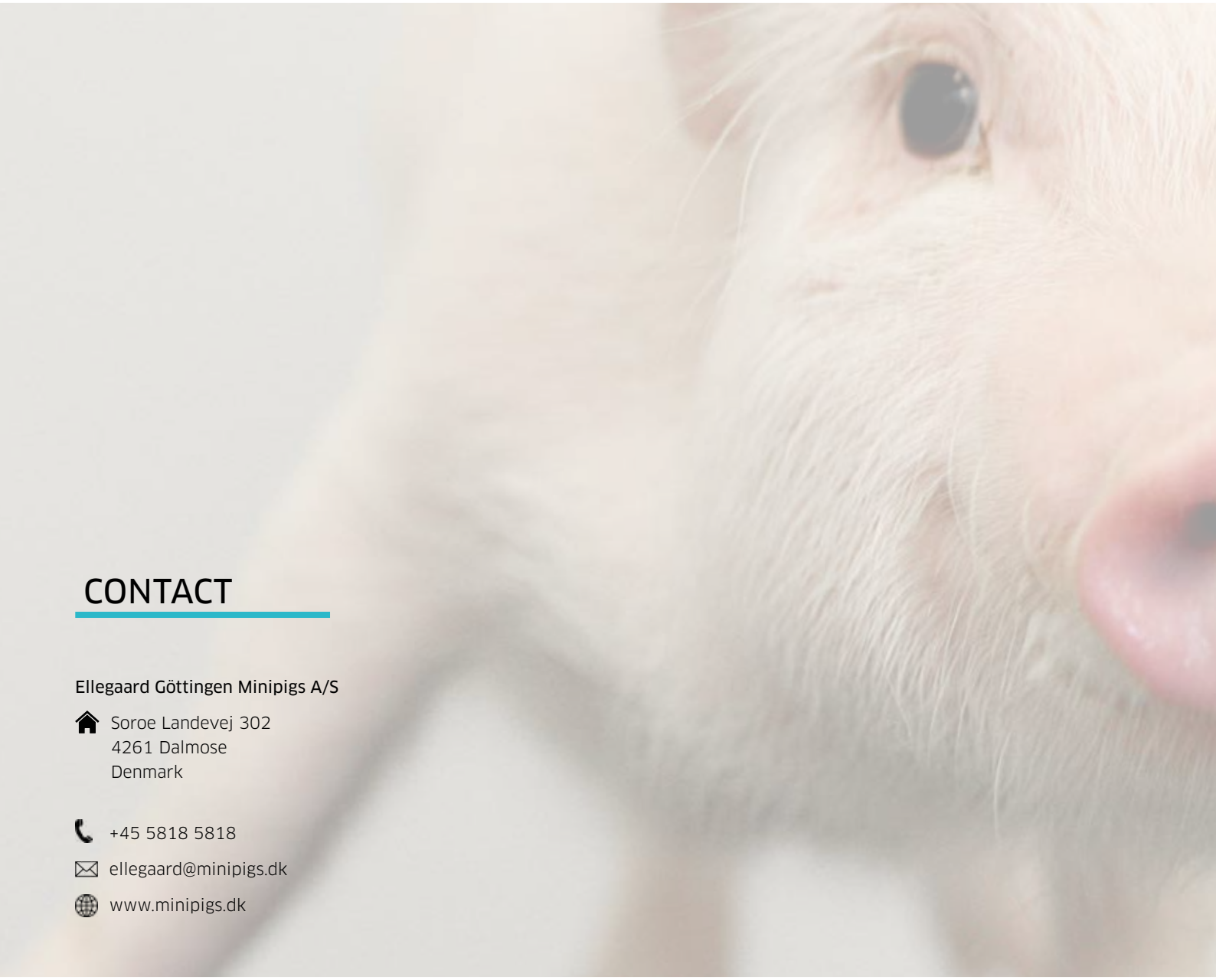
# Göttingen Minipigs
## MAGAZINE

ELLEGAARD
GÖTTINGEN MINIPIGS

# Dear reader

In this edition of the Göttingen Minipigs Magazine, we present new insights to add further to the understanding and application of Göttingen Minipigs in biomedical research. Our focus in this issue is on providing practical resources and insights to support your work with and choice of Göttingen Minipigs as a large animal model.

On pages 4-5 (and on minipigs.dk), you will find a comprehensive resource on Göttingen Minipigs histology and pathology. Whether you are new to using Göttingen Minipigs or have extensive experience, this overview offers a valuable collection of published materials. Utilize it to establish a foundational understanding of Göttingen Minipigs histology and pathology or to discover the



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findings of fellow researchers in specific areas. Delve into the MR/CT imaging atlases of Göttingen Minipigs, including their brain, or refer to the comprehensive work on vehicle systems and routes of administration to enhance your study design.

I am also pleased to announce the Göttingen Minipigs Symposium, scheduled for June 11th, 2024, in Cambridge, MA. This event will feature presentations from leading experts on regulatory acceptability, species selection in drug safety testing, and innovations in nonclinical pharmaceutical development. This symposium is an excellent opportunity to engage with peers and expand your knowledge on the use of Göttingen Minipigs in biomedical research. Find more information on page 29.

At Ellegaard Göttingen Minipigs, we are committed to using sustainable and responsible practices. Our efforts reflect our dedication to reducing environmental impact and promoting energy efficiency within our operations. Read more on page 28.

Finally, we invite EUROTOX participants to visit our facilities as part of their trip to Copenhagen. This tour includes an introduction to our company, a viewing of our Göttingen Minipigs, and presentations on their use in toxicology and future developments. On page 25, you can see how to join the trip.

I hope this edition provides valuable insights and resources to support your research and application of Göttingen Minipigs. Thank you for your continued engagement and support.

*Martin Windfeld Velin, CEO*
*Ellegaard Göttingen Minipigs A/S*

# CONTENTS

# Build or expand your knowledge on Göttingen Minipigs histology and pathology

**A new page collecting available material published on Göttingen Minipigs, proving their relevance as a non-rodent animal species in biomedical research, has become available.**

Whether you are new to Göttingen Minipigs as a large animal model or have years of experience working with them, you might find this page interesting and useful. Use it to build your basics on histology and pathology or learn about fellow scientists' findings within specific areas of research. Explore the MR/CT imaging atlases of Göttingen Minipigs and their brain. Or use the reference work for vehicle systems and routes of administration to help build your study.

## The RETHINK project

The RETHINK project, is a comprehensive initiative aimed at evaluating the use of minipigs in regulatory toxicity testing. The project assembled expert study groups to review five different areas relating to the use of minipigs in regulatory safety testing. The findings suggested that there were no specific areas where restrictions to the use of minipigs in toxicology were required for welfare reasons. The minipig model was generally acceptable to regulatory authorities, provided it was adequately justified. The minipig was found to be an interesting model for safety testing due to numerous anatomical, physiological, genetic, and biochemical similarities to humans. In addition, many features of the minipig made it a practical and flexible model for safety testing.


## Data for download

The following parameters in Göttingen Minpigs have been recorded and is available for download:

- Blood data on IgG Humanized Göttingen Minipigs
- Clinical chemistry
- Growth curve
- Growth data
- Hematology and hemodynamics
- Histopathology
- Organ weights

## Pathological background data

- The incidence of congenital malformations and variations in Göttingen minipigs
- Pigs in Toxicology: Breed Differences in Metabolism and Background Findings
- A Brief Review of Infrequent Spontaneous Findings, Peculiar Anatomical Microscopic Features, and Potential Artifacts in Göttingen Minipigs
- Background Pathological Changes in Minipigs: A Comparison of the Incidence and Nature among Different Breeds and Populations of Minipigs
- Spontaneous Background Pathology in Göttingen Minipigs
- Spontaneous lesions in clinically healthy, microbiologically defined Göttingen minipigs

## *Easy access*

Follow the link below for the full overview listed on these pages (preview to the right):

minipigs.dk/about-gottingen-minipigs/background-data

Remember to add the page to your favourites for easy access in the future.


## Atlases of CT/MRI scans

Gain access to detailed CT/MRI scans of Göttingen Minipigs from both sexes and at different ages enabling the opportunity to follow the development of all organs over time and obtain new data.

A comprehensive imaging atlas presents detailed information about the physiology of Göttingen Minipigs has been made available for anyone interested in the anatomical evolution of Göttingen Minipigs.

A comprehensive selection of histological sections of the Göttingen Minipigs brain paired with MRI scans.

## Ressources on formulation and dosing

Learn about practical information on formulation and dosing in Göttingen Minipigs.

**Article: "Vehicle Systems and Excipients Used in Minipig Drug Development Studies"**
This meta article contains a broad catalog of used vehicles and excipients, indexing the findings and plenty of references for further investigation.

**Routes of administration**
A technical guide with the most basal dosing routes in Göttingen Minipigs incl. instructions in training and handling.

## Recommended papers

Download a selection of papers grouped into topics to illustrate areas in which Göttingen Minipigs have excelled.

The list of topics and papers is far from exhaustive but includes a selection of papers with a more general approach, which have contributed to the general understanding and characterisation of Göttingen Minipigs as a large animal model.

The collection includes:

- ADME and PK/PD
- Biomaterial
- Cardiovascular
- Immunology
- Metabolism
- Methods
- Neuro
- Novel Modalities
- Ocular
- Toxicology and Safety

# Advancing Ethical Standards in Minipig Studies: The role of Vascular Access Buttons

*By Mèlanie Reijnaers[1], Joelle van Dijk[1], Gabry Warmels[1], and Judith Latour[1]*

*[1]Charles River Laboratories, The Netherlands*

**Vascular Access Buttons (VAB) are increasingly becoming the preferred method for blood collection in rodent studies, offering a revolutionary approach that minimizes the need for frequent vein punctures. This technique represents a significant advancement in promoting the ethical conduct of animal research by providing a more convenient and less invasive method for intravenous drug administration and blood collection. In addition to their use in rodent studies, there is a growing interest in exploring the applicability of VAB buttons in other animal species. Charles River Laboratories Den Bosch in collaboration with Ellegaard Göttingen Minipigs A/S explored this application in Göttingen minipigs, conducting a study to compare the VAB with the traditional vena cava cranialis puncture. The study meticulously evaluated pharmacokinetic and clinical pathology parameters sampled via both methods, demonstrating their reliability and interchangeability. Furthermore, findings underscored the practicality, durability, and welfare enhancements associated with VAB employment in Göttingen minipigs, suggesting their potential as an ethical and reliable alternative for future research involving intravenous drug administration and blood collection in this species.**

Vascular Access Buttons (VAB) are increasingly becoming the preferred industry standard for repeated blood collection in (extended) in vivo studies in rats and mice. Unlike with conventional vein punctures and vascular access ports (VAP), the VAB introduces a revolutionary approach featuring a self-sealing silicone membrane that eliminates the need for needle access. This sets it apart from VAPs, which relies on needle puncture of a septum-covered port and is therefore not often used as an alternative for conventional vein punctures. Consequently, the innovative VAB technology does allow animal technicians to access the vascular system without the need for frequent vein punctures, thereby minimizing stress and discomfort for the animals involved.

By providing a more convenient and less invasive method for intravenous drug administration and blood collection, the use of VAB buttons aligns with the guiding principles of the 4 R's—Reduce, Refine, Replace, Responsibility. For that reason, this advanced technique signifies a significant leap forward in ensuring the responsible conduct of animal research, emphasizing the ethical imperative of further exploration and investigation into potential applications of the VAB technology.

In addition to their use in rodent studies, there is growing interest in exploring the utility of VAB buttons in other animal species. In current in vivo studies involving Göttingen minipigs, stress remains a critical concern, particularly during the process of restraining, blood collection, and administering drug substances. The repetitive nature of venipunctures for blood collection, especially common in pharmacokinetic studies, exacerbates this issue and can compromise both animal welfare and data integrity. In severe cases, the stress-induced complications may even necessitate euthanasia, emphasizing the need for a more ethical and reliable alternative. Recognizing this need, Charles River Laboratories Den Bosch in collaboration with Ellegaard Göttingen Minipigs A/S have turned to VAB buttons as a potential solution.

A study was conducted at Charles River Laboratories Den Bosch to assess the feasibility, validity, and potential benefits of pre-implanted VAB buttons in Göttingen minipigs compared to the traditional method, the vena cava cranialis (VCC) puncture. By evaluating the reliability and usability of the VAB buttons, especially in the context of pharmacokinetic studies, the study aimed to mitigate stress and discomfort for minipigs while maintaining or, at best, enhancing the accuracy and reliability of study outcomes in future research.

## METHODS

### Surgical Implantation of Vascular Access Buttons in Minipigs

For this study, a surgical procedure was performed on two Göttingen minipigs to implant the VAB button. The left external jugular vein was carefully dissected, and after ligation, two 3fr PU catheters were inserted at intervals of 7 and 9 cm. The catheters were securely fastened around the vessel using a modified Miller knot followed by square knots. Additional ties were placed cranially to the bead, and the catheter was further secured with the ends of the initial ligature. The incision was closed in three layers using PSDII, employing a continuous pattern, with the final layer intradermal. Subsequently, the catheters were tunneled to a subcutaneous pocket created behind the left ear and connected to the button. The implantation site was closed in three layers. Anesthesia was induced using a combination of zoletil, butorphanol, ketamine, and xylazine, and maintained with isoflurane. After discontinuation of anesthesia, the minipigs were allowed to recover. Following one week of post-operative care, including antibiotics and analgesia (a combination of butorphanol and ketamine), vascular access was tested. Meloxicam was administered before surgery and continued for three days post-surgery. The animals were then transferred to Charles River Laboratories Den Bosch.

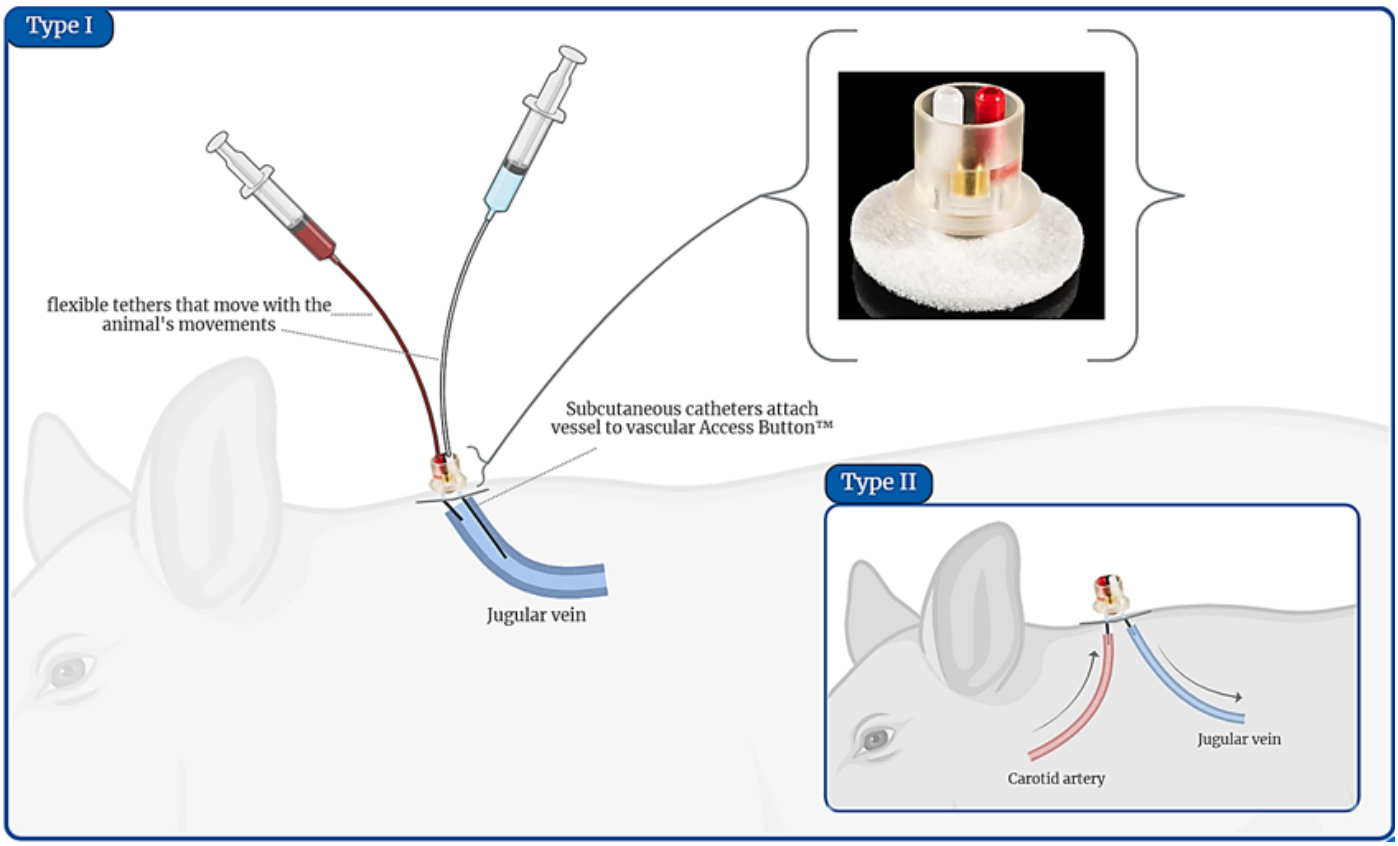


**Figure 1**
**Graphical Representation of the Vascular Access Button (VAB) system**
The figure displays two types of VAB buttons implanted under the skin. One animal had a VAB implemented with two catheters of different length in the jugular vein (Type I) and the other animal had a VAB implemented with two catheters of different length one in the jugular vein and one in the carotid artery (Type II). The subcutaneous catheters attached the vessel to the VAB. The VAB button consisted of two ports, one for drug administration (longer catheter) and one for blood collection (shorter catheter).

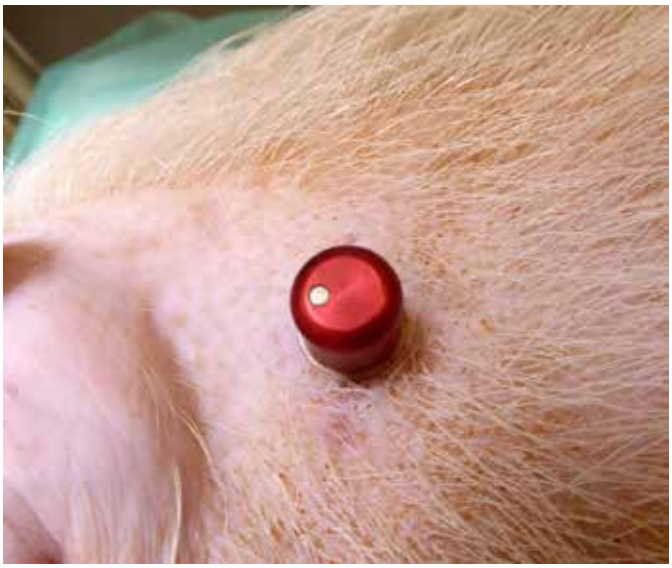

**Photo 1**
Göttingen Minipig with Vascular Access Button behind the ear.
(Photo from Ellegaard Göttingen Minipigs A/S)

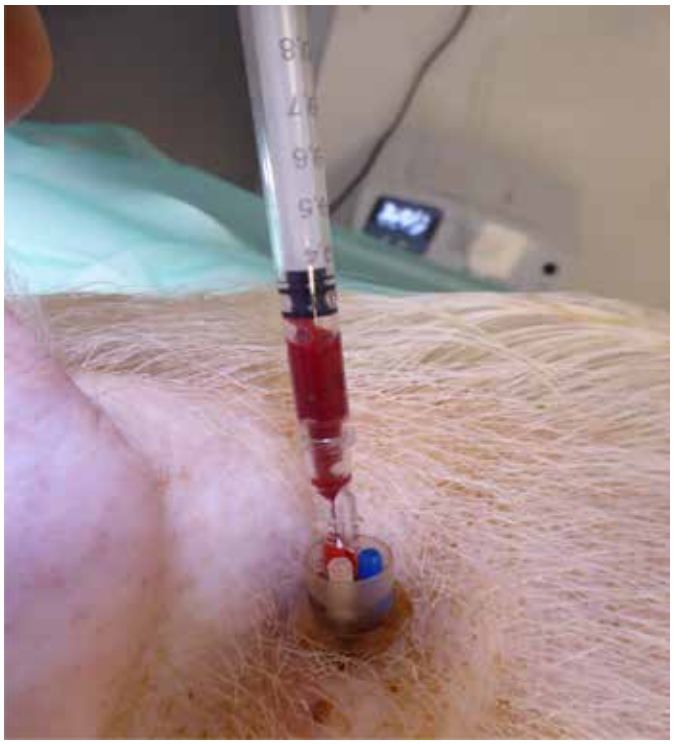

**Photo 2**
Blood sampling from Vascular Access Button.
(Photo from Ellegaard Göttingen Minipigs A/S)

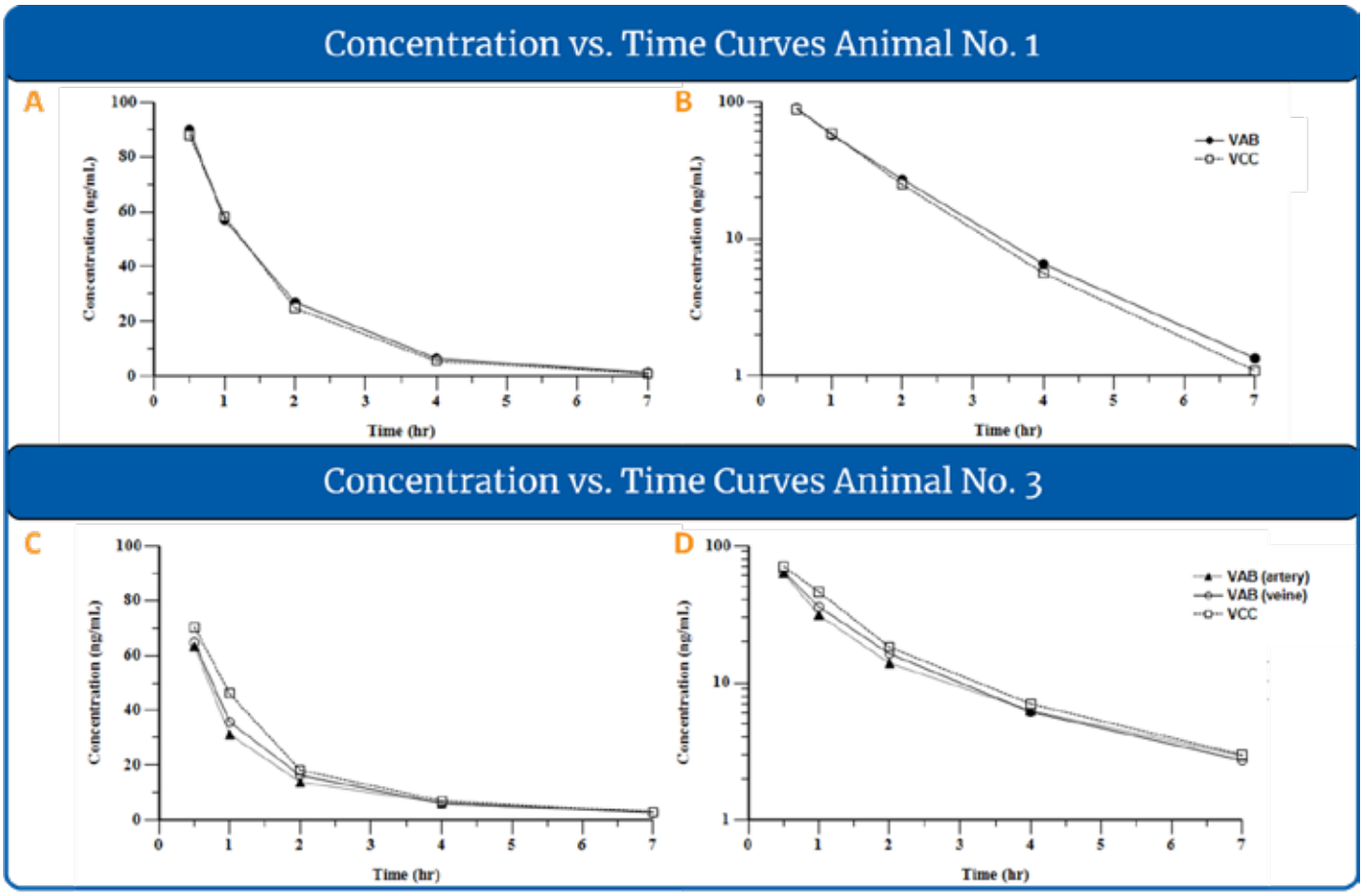


**Figure 2**
Concentration vs. Time curves for Dexamethasone in Animal No. 1 (VAB vs. VCC) and Animal No. 3 (VAB artery vs. VAB vein vs. VCC) following single intravenous injection using the VAB. Graph A and C show the linear:linear curve and Graph B and D show the log:linear curve.

| Animal No. | Sampling technique | $C_0$ (ng/mL) | $C_0$/Dose (ng/mL /(mg/kg)) | $AUC_{tlast}$ (hr*ng/mL) | $AUC_{tlast}$ /Dose (hr*ng/mL /(mg/kg)) | $AUC_{0\text{-}inf}$ (hr*ng/mL) | $AUC_{0\text{-}inf}$ /Dose (hr*ng/mL /(mg/kg)) | $\lambda_z$ (1/hr) | $t_{1/2}$ (hr) | Cl (mL/hr/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | VAB | 142 | 475 | 182 | 608 | 184 | 614 | 0.623 | 1.11 | 1630 |
| | VCC | 133 | 443 | 174 | 579 | 175 | 584 | 0.678 | 1.02 | 1710 |
| 3 | VAB (artery) | 128 | 428 | 128 | 427 | 137 | 457 | 0.308 | 2.25 | 2190 |
| | VAB (vein) | 117 | 391 | 132 | 441 | 140 | 465 | 0.351 | 1.97 | 2150 |
| | VCC | 108 | 359 | 146 | 487 | 154 | 513 | 0.356 | 1.95 | 1950 |

**Table 1**
Pharmacokinetic Parameters of Dexamethasone in Male Göttingen Minipig Plasma after Sampling via Different Techniques Following Single Intravenous (Slow Bolus) Injection Using the VAB

| | | | | Sampling Technique Ratio | |
|---|---|---|---|---|---|
| | | | | $C_0$ | $AUC_{tlast}$ |
| Analyte | Animal No. | Reference | Comparator | | |
| Dexamethasone | 1 | VCC | VAB | 1.07 | 1.05 |
| | 3 | VCC | VAB (artery)<br>VAB (vein) | 1.19<br>1.08 | 0.877<br>0.904 |

**Table 2**
Sampling Technique Ratios of Dexamethasone in Male Göttingen Minipig Plasma after Sampling via Different Techniques Following Single Intravenous (Slow Bolus) Injection Using the VAB

## Ensuring Optimal Proficiency: Training and Maintenance Protocol

At the onset of the study, Ellegaard Göttingen Minipigs provided comprehensive training sessions aimed at ensuring the proficiency of animal technicians in catheter maintenance, intravenous dosing, and blood collection techniques utilizing the VAB. This training regimen spanned a four-week period, during which the animal technicians familiarized themselves in the intricacies of these tasks. Emphasis was placed on the importance of regular catheter maintenance to uphold the optimal functionality of the VAB. This regular catheter maintenance involved weekly flushing using solutions such as Taurolock, heparin/saline, or heparin/glycerol to effectively mitigate the risk of clot formation and to sustain the patency of the catheters. Moreover, daily practice sessions, excluding weekends, were dedicated to honing skills in blood sample collection and intravenous dosing (using saline with a maximum volume of 2.5 mL/kg). Throughout the entire duration of the study, spanning a 120-day period until necropsy, the usability and durability of the VAB was evaluated to ascertain its reliability and longevity in a research setting.

## The Methodological Approach: How we compared the VAB to the VCC puncture

Following the initial phase of training, the study progressed to assess the effectiveness and validity of two distinct VAB buttons implemented in two separate Göttingen minipigs. In one minipig, both catheters of the VAB were surgically inserted into the jugular vein, whereas in the other, a hybrid approach was adopted, with one catheter positioned in the jugular vein and the other placed in the carotid artery [see Figure 1 for a visual representation]. This strategic variation in catheter placement aimed to explore the versatility and usability of the VAB across different anatomical contexts.

Subsequently, dexamethasone, a widely used veterinary pharmaceutical agent, was administered via the VAB to both minipigs at a dose level of 0.3 mg/kg, followed by repeated blood collection for pharmacokinetic parameter analysis via both the VAB button and the traditional method, VCC puncture. It is important to note that blood collection via both methods was meticulously synchronized, ensuring simultaneous sampling to minimize potential confounding factors. This methodological precision was imperative for facilitating accurate comparisons within each minipig between the VAB and the traditional VCC method, as well as between the two distinct VAB buttons employed. Additionally, a comprehensive evaluation of clinical pathology parameters was conducted using blood samples obtained from both the VAB and the VCC for each minipig, prior to dosing, enabling a robust assessment of result consistency and methodological reliability within the study.

## RESULTS

**Question I:** *Comparison of Pharmacokinetic Parameters between the VCC and VAB in Göttingen minipigs.*

**Result:** Table 1 outlines the pharmacokinetic parameters of Dexamethasone in male Göttingen minipigs after a single intravenous (slow bolus) injection via the pre-implanted vascular access button (VAB). The exposure data for Animal No. 1 (with VAB Type I) and Animal No. 3 (with VAB Type II) yielded comparable results within each animal, regardless of the blood collection technique used [Table 1; Table 2]. For Animal No. 1, the sampling technique ratio between the VAB and the VCC was 1.07 for C0 and 1.05 for AUCtlast. For Animal No. 3, the sampling technique ratio between the VAB artery and the VCC was 1.19 for C0 and 0.88 for AUCtlast, while the ratio between the VAB vein and the VCC was 1.08 for C0 and 0.90 for AUCtlast. The consistency of the pharmacokinetic parameters of Dexamethasone across different blood collection techniques is also evident from the individual Concentration vs. Time curves in each Animal [Figure 2].

Thus, the pharmacokinetic parameters in Göttingen minipigs remained consistent across different blood collection techniques, as evidenced by comparable results within each animal. The slight variations observed can be due to normal variation but could also suggest small variations in the time of collection. Overall, the VAB proved to be a reliable method for intravenous drug administration and sampling. These findings support the utility of VAB for pharmacokinetic blood collection in in vivo studies with Göttingen minipigs.

**Question II:** *Comparison of Clinical Pathology Samples from VCC and VAB in Göttingen minipigs*

**Result:** This test aimed to investigate the comparability of clinical pathology samples obtained via the VCC and the VAB in Göttingen minipigs. Hematology, coagulation, and clinical chemistry values were measured and assessed for similarity between the two sampling methods [Table 3-5]. The comparison of clinical pathology parameters revealed consistent findings across most parameters, with only one notable exception. Hematological parameters, including red blood cell count, white blood cell count, and platelet count, showed negligible disparities between the techniques, all falling within clinically acceptable ranges[1]. Coagulation values, i.e., prothrombin time and activated partial thromboplastin time, exhibited similar results. Likewise, clinical chemistry values, encompassing electrolyte levels, liver function markers, and renal function indices, demonstrated comparable outcomes irrespective of the sampling technique employed for all but one parameter. One anomalous result was observed in one animal its clinical chemistry profile; notably elevated triglyceride levels when sampled via the VAB compared to VCC. This difference however is supported by literature that states how hugely triglycerides can vary depending on the location the blood is sampled; especially the conventional VCC tends to give an underestimate result for triglycerides[2]. For that reason, a crucial point to bear in mind is to standardize the site of collection throughout the study in order to decrease the potential for errors in data interpretation. The minor variations observed in all other clinical chemistry parameters were within acceptable limits and likely attributable to inherent biological variability.

In summary, while variations were observed between the two sampling locations, the overall congruity between samples collected via VCC and VAB suggests their interchangeability for clinical pathology analysis in Göttingen minipigs. The demonstrated equivalence between the VCC and VAB confirms that researchers can confidently utilize the VAB technique for blood sample collection for clinical pathology in Göttingen minipig studies.

| Hematology Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Hematology Parameter | | EOS (10^9/L) | HCT (L/L) | HGB (g/L) | LUC (10^9/L) | LYMPH (10^9/L) | MCHC (g/L) | MCH (pg) | MCV (fL) |
| Animal No. 1 | VAB | 0.18 | 0.446 | 149 | 0.10 | 6.92 | 335 | 16.8 | 50.2 |
| | VCC | 0.20 | 0.488 | 165 | 0.12 | 7.87 | 337 | 17.0 | 50.4 |
| Animal No. 3 | VABa | 0.12 | 0.431 | 146 | 0.17 | 5.92 | 340 | 17.9 | 52.5 |
| | VABv | 0.10 | 0.434 | 145 | 0.18 | 5.62 | 335 | 17.6 | 52.5 |
| | VCC | 0.13 | 0.456 | 153 | 0.22 | 6.73 | 335 | 17.7 | 52.7 |
| Hematology Parameter | | MONO (10^9/L) | NEUT (10^9/L) | PLT (10^9/L) | RBC (10^12/L) | RDW (%) | RETIC (10^9/L) | WBC (10^9/L) | |
| Animal No. 1 | VAB | 0.29 | 1.98 | 336 | 8.89 | 14.9 | 18.8 | 9.49 | |
| | VCC | 0.34 | 2.20 | 353 | 9.69 | 15.1 | 37.9 | 10.76 | |
| Animal No. 3 | VABa | 0.19 | 3.98 | 386 | 8.20 | 15.6 | 33.4 | 10.41 | |
| | VABv | 0.19 | 3.80 | 393 | 8.27 | 16.0 | 39.5 | 9.91 | |
| | VCC | 0.28 | 4.16 | 291 | 8.66 | 15.9 | 64.4 | 11.56 | |

**Table 3**
Hematology Parameters of Male Göttingen Minipigs after Sampling via Different Techniques.

| Clinical Chemistry Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Clinical Chemistry Parameter | | ALT (U/L) | AST (U/L) | ALP (U/L) | TBIL (µmol/L) | GGT (U/L) | CK (U/L) | UREA (mmol/L) | CREAT (µmol/L) | CA (mmol/L) | PHOS (mmol/L) | TPROT (g/L) |
| Animal No. 1 | VAB | 68 | 37 | 165 | 1.8 | 67 | 330 | 2.7 | 84 | 2.77 | 2.11 | 66.9 |
| | VCC | 69 | 59 | 171 | 2.2 | 69 | 339 | 2.6 | 73 | 2.79 | 2.18 | 68.1 |
| Animal No. 3 | VABa | 46 | 20 | 129 | 1.4 | 34 | 383 | 1.3 | 60 | 2.64 | 1.96 | 57.4 |
| | VABv | 46 | 22 | 132 | 2.1 | 35 | 397 | 1.3 | 61 | 2.65 | 1.96 | 58.0 |
| | VCC | 46 | 26 | 127 | 1.5 | 36 | 505 | 1.3 | 65 | 2.63 | 1.82 | 54.1 |
| Clinical Chemistry Parameter | | ALB (g/L) | GLOB (g/L) | A/G (ratio) | GLUC (mmol/L) | CHOL (mmol/L) | TRIG (mmol/L) | NA (mmol/L) | K (mmol/L) | CL (mmol/L) | PLIP (mmol/L) | LDH (U/L) |
| Animal No. 1 | VAB | 49.8 | 17.1 | 2.9 | 4.52 | 1.44 | 0.36 | 147 | 4.7 | 104 | 1.26 | 437 |
| | VCC | 52.7 | 15.4 | 3.4 | 5.87 | 1.51 | 0.29 | 146 | 5.1 | 104 | 1.31 | 661 |
| Animal No. 3 | VABa | 45.6 | 11.8 | 3.9 | 5.02 | 1.51 | 1.50 | 142 | 4.4 | 104 | 1.33 | 383 |
| | VABv | 45.7 | 12.3 | 3.7 | 4.83 | 1.39 | 2.37 | 143 | 4.4 | 102 | 1.34 | 395 |
| | VCC | 43.4 | 10.7 | 4.1 | 7.11 | 1.33 | 0.23 | 145 | 5.1 | 105 | 1.36 | 447 |

**Table 5**
Clinical Chemistry Parameters of Male Göttingen Minipigs after Sampling via Different Techniques.

| Coagulation Parameters | | | |
|---|---|---|---|
| Coagulation Parameter | | PT (sec) | APTT (sec) |
| Animal No. 1 | VAB | 15.0 | 20.2 |
| | VCC | 14.1 | 16.8 |
| Animal No. 3 | VABa | 15.2 | 17.8 |
| | VABv | 15.3 | 17.9 |
| | VCC | 15.5 | 18.8 |

**Table 4**
Coagulation Parameters of Male Göttingen Minipigs after Sampling via Different Techniques.

**Question III:** *How practical is the use of the VAB button and for what duration can it be used?*

**Result:** Both Göttingen minipigs exhibited no signs of disruption to their VAB button and/or catheter from (group)-housing conditions, nor was there any appearance of seromas surrounding the VAB button due to the surgical procedure. The animals were successfully housed together for a period of 53 days, which was made possible due to the protective aluminum cap on the button. It is important that this button is securely fastened to prevent loss in the cage.

Furthermore, the system improved the ease of blood collection by requiring only a PinPortTM injector attached to a syringe, allowing blood collection by a single animal technician without additional animal restraint. The VAB system remained patent throughout the entire 120-day study period for both animals. It is worth noting that the animal technicians applied positive pressure on the lock solution syringe during removal from the VAB septum to minimize the risk of clot formation. In cases of initial blood collection failure (four occurrences), an extra flushing procedure was performed, resolving the issue in all instances.

The results highlight the resilience and practicality of the VAB system in Göttingen minipigs, demonstrating its effectiveness in facilitating blood collection while ensuring the animals' welfare during group housing conditions. The catheters attached to the VAB prevented damage by the animals, due to their secure and self-contained design. Overall, we demonstrated that the VAB system offers a practical, durable, and efficient solution for blood sample collection and drug administration in Göttingen minipigs. Its ability to mitigate stress from the animals, reduce procedural time, and require minimal technical support positions it as a valuable technique for future research.

## CONCLUSION

The Vascular Access Button (VAB) has generally proven to be a reliable and valuable alternative for intravenous drug administration and blood collection in Göttingen minipigs, particularly in research or clinical settings requiring frequent blood sampling. However, akin to any medical device, its reliability hinges on factors such proper placement, maintenance, and user proficiency. Adhering to manufacturer guidelines and best practices is crucial to ensure consistent and accurate blood collection. For instance, applying positive pressure to the lock solution syringe while disengaging it from the VAB septum minimizes the risk of clot formation. Moreover, utilizing sterile, pharmaceutical-grade lock and flush solutions, sterile catheters and supplies, along with maintaining aseptic technique during both surgical procedures and catheter maintenance must remain paramount. The use of non-sterile solutions can lead to patency issues at a minimum, or worse infections that could compromise the research integrity and the well-being of the animals.

Overall, the exploration of the VAB technology signifies a promising path toward elevating ethical standards in animal research. By prioritizing the welfare of research subjects and refining experimental methodologies, VAB buttons have the potential to revolutionize the field of in vivo studies across various non-rodent species. As such, continued research and innovation in this domain are essential to realizing the full benefits of this technology.

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2 Neptun DA, Smith CN, Irons RD. Effect of sampling site and collection method on variations in baseline clinical pathology parameters in Fischer-344 rats. 1. Clinical chemistry. Fundam Appl Toxicol. 1985 Dec;5(6 Pt 1):1180-5. doi: 10.1016/0272-0590(85)90155-1. PMID: 4092880.

# Insights from an Alternative Administration Procedure in the Göttingen Minipig: When Innovation Meets Expertise

*By Giuseppe Sacco[1#], Cecile Sobry[2#], Paola Minosi[1], Emilia Carbone[1], Andrea Parenti[1], Vincenzo Pentella[1], Cristina Longobardi[1], Silvana Venturella[1], Daniela Gallo[1*]*

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*[#]Equally contributed to this paper*
*[*]To whom correspondance should be addressed: Daniela Gallo, Toxicology & Pathology Sciences Director, ERBC Group, dgallo@erbc-group.com*

**Intravesical drug delivery (IDD), involving placement of drugs directly into the bladder through a urethral catheter, is commonly used to treat various bladder diseases including bladder cancers, urinary tract infections and interstitial cystitis. This regional therapy thus provides a high concentration of drugs to the diseased bladder, with low risk of systemic side effects. Here, we describe the use of Göttingen Minipig as a model for the assessment of local tolerance/toxicity, following repeated intravesical instillation of new drugs. Although challenging to perform and requiring significant technical skills, the procedure was shown to be overall safe and well tolerated.**

## Introduction

The minipig experimental model has become increasingly used in the last few years, as an alternative to the more traditional dog and nonhuman primate (NHP) non-rodent species (1). Indeed, when compared to other non-rodent species, the closer correlation with humans from anatomical, physiological and biochemical points of view makes the minipig model an excellent candidate for translational research (2). Specifically, pigs appear to be a more suitable model than dogs for immunological evaluation (3) and they have been used in investigations on cardiovascular and respiratory safety pharmacology (4, 5), as well as in studies of dermal (6), neurological, reproductive and other different pathologies (7, 8). Besides, their relative small size allows easier handling procedures, together with a reduction in the amount of food, housing space and test item needed to carry out a study. This model is therefore generally accepted by regulatory authorities, provided it is adequately justified (9). Although several breeds of minipigs have been produced, among these, Göttingen Minipigs are the most used for biomedical purposes (10, 11).

The possibility to perform and exploit almost any type of route of administration, constitutes another important feature of this non-clinical model that reinforces its translational power (12). In this regard, besides common routes of administration (e.g. oral, intravenous, subcutaneous, dermal and intramuscular), other unusual ones can be exploited to perform toxicology studies in minipigs, including intravitreal, intratracheal, intraarticular, intranasal and intravesical (13, 14).

Specifically, intravesical drug delivery (IDD), providing a high concentration of drugs to the diseased bladder, represents a viable approach for treating various bladder diseases including bladder cancers, urinary tract infections and interstitial cystitis (15, 16). Indeed, owing to its anatomical features, urinary bladder represents an effective barrier that prevents the diffusion of toxic substances from the urine into the bloodstream, this reducing the risk of systemic side effects after local drug administration (17).

**Figure 1**
The picture shows the pre-anesthesia procedure performed by a veterinarian. Animals, fasted for about 12 hours before administration, were premedicated with a mixture of Ketamine, Xylazine and Diazepam (5-10 mg/kg, intravenous) and maintained by Isoflurane (1.5-3%) inhalation (Oxygen flow 1.5-2 L/min).

Due to the similarity to humans with respect to the urogenital anatomy, urine density, and immune system physiology (18), pig has proved to be a suitable model for non-clinical studies involving IDD. Importantly, pigs are natural host of uropathogenic *Escherichia coli* (UPEC), the predominant etiologic agent of human urinary tract infections (UTIs) (14, 19).

Here we present a detailed protocol for tolerance/toxicity studies after repeated urinary bladder instillation in Göttingen Minipigs. Only female minipigs are used since a retrograde approach to the bladder through the urethra is not possible in males due to their penile anatomy (sigmoid flexure of the penis).

## Materials and methods

Six Göttingen Minipig females, approximately 4-5 months old and weighing 9-11 kg, were purchased from Ellegaard Göttingen Minipigs (Dalmose, Denmark), as part of a batch of 26 female Göttingen minipigs, to be used in a repeated dose toxicity study. These animals acted as controls in a regulatory toxicity study carried out at European Research Biology Center (ERBC) S.r.l., (Pomezia, Rome, Italy), a GLP certified facility. Procedures and facilities were compliant with the requirements of the Directive 2010/63/EU on the protection of animals used for scientific purposes. The national transposition of the Directive is defined in Decreto Legislativo 26/2014. ERBC is fully accredited by Association for Assessment and Accreditation of Laboratory Animal Care International and the aspects of the protocol concerning animal welfare were approved by the animal welfare body.

The animals were group housed (two animals per pen) in indoor enclosures of approximately 200×135 cm floor size both during the acclimatization period and experimental phases. Single housing was limited to the periods necessary for a correct performance of animals care and experimental procedures. Drinking water was offered *ad libitum* throughout the study, and a weighed amount of diet [Altromin 9069, Maintenance diet for minipig, Altromin Spezialfutter GmbH & Co. KG ImSeelenkamp 20, 32791 Lage, Germany] was offered daily divided in two rations.

Animals, fasted for about 12 hours before administration, were premedicated with a mixture of Ketamine (10 mg/kg, intramuscular), Xylazine (1 mg/kg, intramuscular) and Diazepam (5-10 mg/kg, intravenous, if necessary) and maintained by Isoflurane (1.5-3%) inhalation (Oxygen flow 1.5-2 L/min) (Fig. 1). Following the pre-anesthesia, each animal was subjected to gaseous anesthesia by means of a mask (2-5% Isoflurane), then intubated through an orotracheal tube and maintained under gaseous anesthesia (1.5-3% Isoflurane) for the whole treatment period (approximately 1-hour exposure). The anaesthetized animal was placed onto the operating table in sternal recumbency. Once the desired stage of anesthesia was achieved, the perineum and vulva were washed and cleaned thoroughly with septal scrubs and a pre-perforated surgical drape was placed. The Foley catheter (Fig. 2), embedded with lubricant gel, was gently introduced into the urethra until the urine flowed into it. A speculum was used to facilitate the introduction of the catheter. The balloon was inflated with approximately 5 mL of sterile saline solution in order to avoid accidental removal of the catheter. When all the urine was removed by gravity, the animal was dosed. A total of 100 mL of sterile physiological saline (negative control) was introduced through the catheter into the urinary bladder followed by 1.5 mL of sterile physiological saline and left in place for an exposure period of 1 hour. At the end of the exposure period, the Foley catheter was removed after the emptying of the urinary bladder. Animals were dosed once weekly for a total of four times, on Days 1, 8, 15 and 22

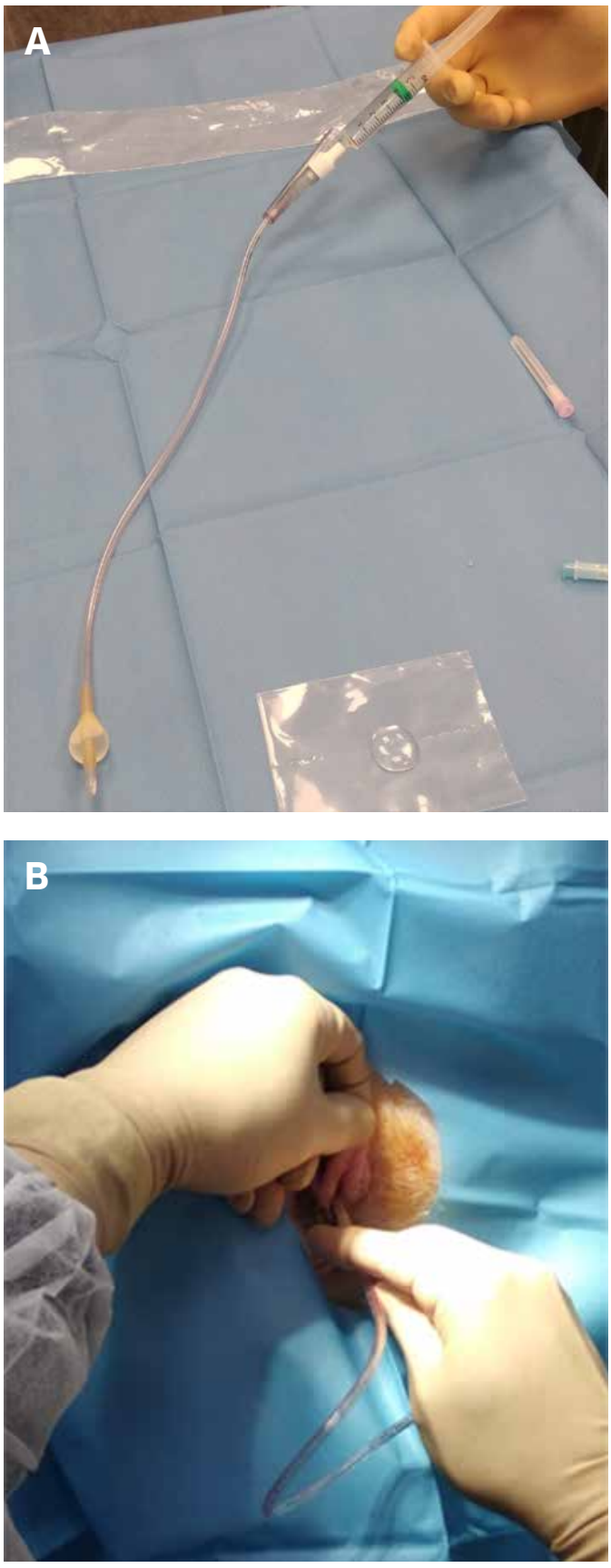


**Figure 2**
Intravesical drug delivery technique in female Göttingen Minipigs. Once the desired stage of anesthesia was achieved, the perineum and vulva were washed and cleaned thoroughly with septal scrubs and a pre-perforated surgical drape was placed. A sterile Foley catheter **(A)** (Teleflex soft simplastic 2-way foley cylindrical tip 12-14 Ch 40 cm length), embedded with lubricant gel, was gently introduced into the urethra **(B)**.

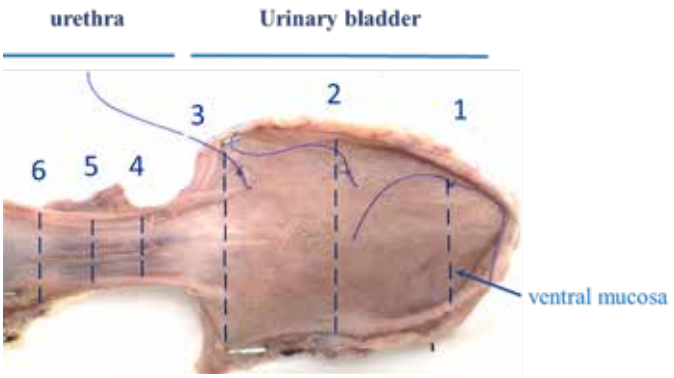


**Figure 3**
At autopsy, tissue samples for histopathological examination of the urinary bladder and Urethra were collected. The organ was opened in the dorsal region from the Urethra, placed on a rigid cardboard and pinned at its borders, to keep it flat during formalin fixation. Six formalin-fixed samples were taken as shown in the photo: 3 in the urinary bladder at the middle (ventral) position in the apical, middle and caudal area (Levels 1, 2, 3) and 3 in the Urethra apical, middle and caudal area (Levels 4, 5, 6).

of the study (4 weeks). Animals in the main phase (4 females) were euthanized the day after last dosing. A recovery period of 2 weeks was allowed after last administration for 2 females.

*In vivo* observations including daily clinical signs, body weight, food and water consumption, physical examination, electrocardiography (ECG) and clinical pathology investigations (including urinalysis) were carried out throughout the observation period.

Immediately after autopsy, tissue samples for histopathological examination of the urinary bladder and urethra were collected. The urinary bladder was opened in the dorsal region from the urethra, placed on a rigid cardboard and pinned at its borders, to keep it flat during formalin fixation.

Six formalin-fixed samples were taken (Fig. 3): 3 in the urinary bladder at the middle (ventral) position in the apical, middle and caudal area (Levels 1, 2, 3) and 3 in the urethra apical, middle and caudal area (Levels 4, 5, 6). Tissues were embedded in paraffin wax, the blocks were sectioned at 5 µm thickness and routine hematoxylin and eosin (H&E) staining was performed. A microscopic examination was performed by a board-certified veterinary pathologist, on the full list of tissues including the urinary tract (kidneys, ureters, urinary bladder, and urethra) for main animals and on the urinary bladder and urethra for recovery animals. The scoring of the lesions was done semi-quantitatively, using a 5-point grading scale for all reported microscopic findings (1= minimal, 2= mild, 3= moderate, 4= marked, 5= severe).

## Results and discussion

All minipigs evaluated in this study were considered healthy on the basis *in vivo* observations, including body weight, food and water consumption, physical examination, clinical pathology analyses, and ECG. There were no relevant changes of parameters evaluated at the end of the dosing and recovery periods compared to pre-dose results.

At necropsy, there were no macroscopic observations in the urinary bladder and urethra at the end of the dosing period. At the end of the recovery period, a single pale area was noted in the urinary bladder of one animal but in the absence of microscopic correlates, it was not considered to be relevant.

Procedure-related microscopic findings were observed in the urinary bladder and urethra. At the end of the dosing period, they consisted of minimal or mild, multifocal mixed inflammatory cell infiltrates in the urothelium and lamina propria of the urinary bladder of 2/4 animals (Fig. 4) and/or urethra of 3/4 animals (Fig. 5), and minimal focal hemorrhage in the urinary bladder or urethra (2/4 animals). Mixed cell infiltrates, affecting all levels examined in both tissues, were composed of mononuclear cells and neutrophils in variable proportion, with higher numbers of neutrophils present in the urethra compared to urinary bladder. A single animal did not show any inflammatory infiltrate in the urinary bladder or urethra. The few microscopic findings noted in the kidneys of main animals were common background

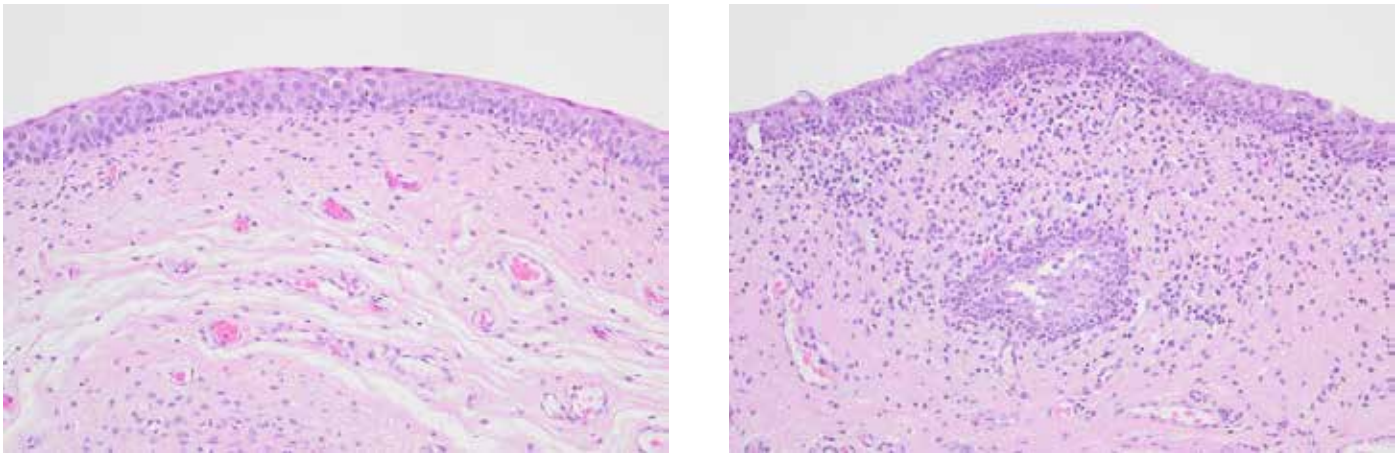

**Figure 4**
Urinary bladder: On the left, unaffected mucosa showing the superficial transitional epithelium (urothelium) above loose collagenous tissue (lamina propria). On the right, infiltrate of mixed inflammatory cells (mononuclear cells mainly) in the urothelium and lamina propria. (H&E, objective 20x).

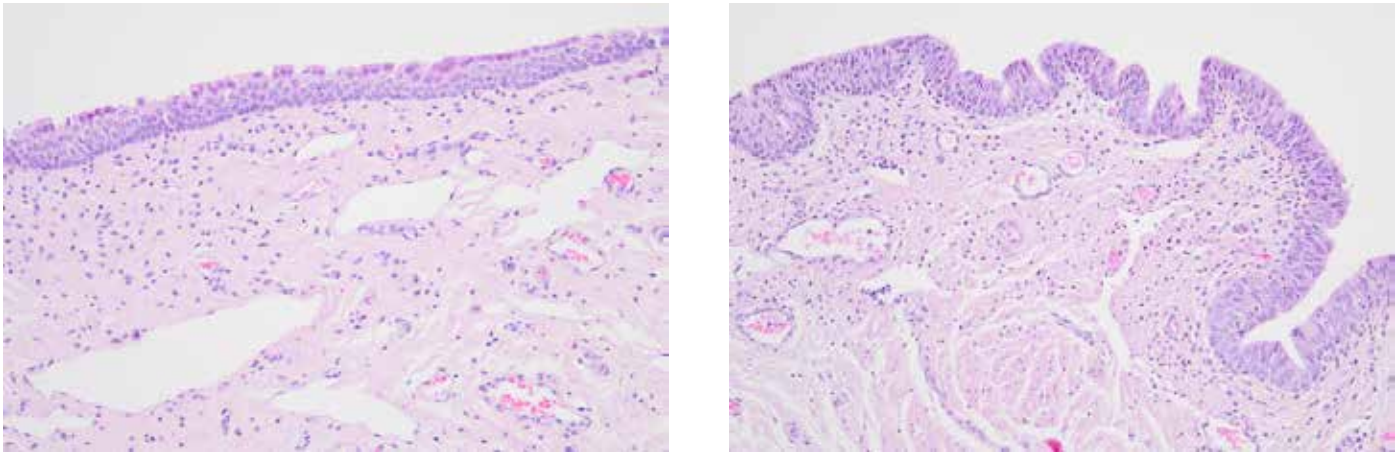

**Figure 5**
Urethra: On the left, unaffected mucosa showing the superficial transitional epithelium (urothelium) above loose collagenous tissue (lamina propria). On the right, infiltrate of mixed inflammatory cells in the urothelium (neutrophils mainly) and lamina propria. (H&E, objective 20x).

findings for the minipig and were not considered to be related to the procedure (20). There were no microscopic findings in the ureters. At the end of the recovery period, minimal or mild, multifocal mixed inflammatory cell infiltrates were still observed in both animals, in the urinary bladder only, in association with minimal multifocal urothelial hyperplasia in one of them. There was full reversibility of the microscopic changes in the urethra.

Results obtained in this study demonstrate that the Göttingen Minipig is an excellent non-rodent animal model for the assessment of local tolerance/toxicity, following repeated (once per week and up to four weeks) intravesical instillation of new drugs. The multiple sampling in specific anatomic regions of the urinary bladder allowed proper histopathological evaluation of any potential findings related to the treatment procedure. Low grade inflammation, hemorrhage and/or reactive urothelial hyperplasia in the urinary bladder and urethra were observed and were considered secondary to repeated microtrauma of the urinary mucosa during catheterization. This points out the need of incorporating negative controls in toxicological studies using this route of administration in order to differentiate potential histopathological changes induced by test items or placebo from procedure-related effects.

On the whole, these results provide evidence for the use of the intravesical administration procedure as safe and well tolerated in this animal model, with procedure-related changes detectable in the urinary bladder and urethra at the microscopic level only. While the procedure is safe and well tolerated, it is challenging to perform and involves significant technical skills by the veterinary staff performing it.

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# REFINED MANAGEMENT AND CARE OF NEWBORN GÖTTINGEN MINIPIGS

**Tania Panfilova, Carina Anker, Maja Ramløse & Kirsten Rosenmay Jacobsen**
**Ellegaard Göttingen Minipigs A/S**

## ABSTRACT

Through early intervention immediately after birth it is possible to reduce the mortality rate among and improve the welfare of newborn piglets. Good results can be achieved through refinement of common procedures in the farrowing stable and for the newborn piglets in e.g. juvenile toxicology and safety studies. This can be through measures like administration of colostrum to weak-born piglets, keeping the piglets warm, and sports tape on the forelegs to reduce the occurrence of leg wounds sustained during suckling. Furthermore, socialization of the sow before and after farrowing creates a calm and trusting environment even when staff members are interacting with the piglets immediately after farrowing. An optimized program for the care of newborn piglets will improve the health and welfare for the animals, thus, building a solid foundation for the minipigs to grow and thrive.

## COLOSTRUM

Consumption of sufficient amounts of colostrum within the first few hours of life is essential for survival and subsequent well-being of newborn piglets (Le Dividich et. al., 2005; Quesnel et. al, 2012). 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 the time, as larger amounts increase the risk of accidental inhalation and subsequent risk of pneumonia. In addition to hand feeding colostrum, we recommend that the piglets have access to the sow as much as possible.

At Ellegaard Göttingen Minipigs, colostrum is kept frozen for up to three months, but studies have shown that human colostrum can be kept frozen for at least six months without losing its immunological properties (Ramírez-Santana et.al., 2012). The colostrum is thawed and heated to 37 °C before administration. Although parity has no effect on the concentrations of immunoglobulins in colostrum (Segura et. al., 2020), colostrum is only collected from multiparous sows (2nd litter and onwards), since primiparous sows yield less amounts of colostrum (Nuntapaitoon et. al., 2020). Furthermore, it is important to eliminate potential stress factors (such as milking) for the first time moms.

## HEAT

If the piglets are cold, it may reduce their ability to absorb antibodies in the gut and they will need to mobilize extra energy reserves (glycogen) to raise their body temperature and maintain metabolism (Villanueva-García et al., 2020). Thus, keeping the piglets warm will increase survival rate. This can be achieved by placing weak-born piglets in a polystyrene box under the heating lamp. The piglets will jump out of the box by themselves when they are warm and strong enough. If piglets are removed from the sow, they must be kept warm, for example by placing an electric heating blanket (31-35°C) or a soft warming cover in the box the piglets are transported in.

## LEG WOUNDS SUSTAINED DURING SUCKLING

It is very common that piglets develop leg abrasions during suckling within the first days of life. The majority are already present within the first 12 hours where suckling is intense and the skin very delicate. At Ellegaard Göttingen Minipigs, we have tested several procedures to reduce the occurrence of these lesions. The most efficient method was to apply a double layer of sports tape (Leukoplast®) on the dorsopalmar surface between the carpal- and fetlock joint on both forelegs. The sports tape comes off by itself in 7-10 days if not removed earlier manually. The tape protects the skin without interfering with normal gait, and still allows the skin to naturally thicken and develop a protective callus. Furthermore, it is an easy procedure that can be done at the first interaction with the piglet.

## TAKING GOOD CARE OF THE SOW– BEFORE AND AFTER FARROWING

Socialization of the pregnant sow for example through handfeeding, petting/scratching or short training sessions based on positive reinforcement (clicker training) before and after farrowing creates a calm and trusting environment, even when staff members are interacting with the piglets or collect colostrum immediately after farrowing. The temperament of the sow influences the piglets and their reaction to humans, and a calm sow typically fosters calm piglets. It should be noted though, that some level of protective maternal behavior is beneficial for the sow's caring for the 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.

## CONCLUSION

Through early administration of colostrum and heat to weak-born and cold piglets we have been able to significantly reduce the mortality rate within this subgroup of piglets. Furthermore, the use of a double layer of Leukoplast® on the forelegs reduced the occurrence of leg wounds sustained during suckling while still allowing the skin to naturally thicken and creating a strong skin barrier during the entire suckling period. And importantly; with a commitment and focus on socialization of the sows, it is possible to create a calm and trusting environment for both the sows, staff and the piglets. This enables staff members to interact with the sow and piglets immediately after farrowing e.g. for veterinary treatment, dosing, sampling, or other types of management interventions.

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ELLEGAARD
GÖTTINGEN MINIPIGS

# Social housing of Göttingen Minipigs

*By Maja Ramløse*[1]

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**Göttingen Minipigs are social and curious animals with a strong motivation for performing various types of social behaviour. Appropriate social housing should therefore be prioritized and unnecessary regrouping should be avoided.**

## Forming new groups

Changes in group composition, such as removal or addition of animals, can lead to changes in group dynamics and instigate the formation of new hierarchies. However, keeping minipigs in stable groups throughout their lives is not always possible due to logistical or study circumstances.

Regrouping of animals is performed successfully on a regular basis at Ellegaard Göttingen Minipigs. Regrouping females can be done at all ages, whereas fighting tendencies amongst males increase in severity with age. Hence, we do not recommend regrouping male minipigs older than approximately 6 months of age. In general, several measures can be taken to support smooth hierarchy and stable group formation. We suggest taking the following into consideration when regrouping minipigs:

Pen prepared to receive regrouped minipigs with straw and scattered food pellets, ice blocks with food pellets, and a selection of toys.

Regrouped minipigs investigating their new pen. The open hatch makes it possible for subordinate minipigs to retract.

*Ease the transition*

If possible, acquaint the minipigs beforehand by housing them in adjacent pens before regrouping them in the same pen.

*Pen area*

If possible, regroup pigs in neutral pens, i.e., pens of which none of the pigs have affiliations or territoriality.

*Space*

Ample space and possibly visual barriers in the pen ensure less stress when forming new hierarchies. An important part of establishing a hierarchy is the possibility for the submissive pig to retract from the confrontation. Adjacent pens and/or the corridor can be used if necessary. Once the hierarchy is established, the pen size can be reduced if necessary.

*Distractions*

Provide plenty of distractions: Bedding such as straw, food scattered on the floor, ice blocks, toys, etc. We find that stimulating the olfactory sense, e.g. with scented toys, works particularly well in alleviating the severity/duration of hierarchal fighting.

*Supervision*

Regrouping should be performed under close observation by staff to enable intervention if hierarchal fighting is prolonged or severe. Hence, regrouping early in the workday is recommended.

*Compatibility*

Determining the social ranking of a minipig in the group can be challenging, but if known, it is easier to regroup a dominant and a submissive minipig, rather than two dominant minipigs.

### Best practice

At Ellegaard Göttingen Minipigs animals are raised in groups. At weaning, animals are split into same-sex groups in new pens, where a social hierarchy is quickly established, after which the groups can remain stable for months.

Animals of all ages are group housed in same-sex groups of at least two minipigs as a standard at Ellegaard Göttingen Minipigs.

Neighbouring pens allowing snout contact and socialisation with other minipigs.

Göttingen Minipigs walking the corridor for stimuli, exercise and meeting other minipigs.

### Reach out to a specialist

Our veterinarian and animal welfare technician are always available for questions and advice in regard to social housing or creating suitable enrichment programs for minipigs at your facilities.

Contact veterinarian@minipigs.dk with your enquiry.

## When is single-housing necessary?

Exceptions to the principle of social housing are animals where veterinary or study needs dictate otherwise, breeding sows where farrowing is imminent, and adult breeding boars, who have a solitary nature. However, even adult boars may be housed together in groups if they remain in the same group they were raised in, under careful supervision of group dynamics.

## Socialising measures for single-housed minipigs

If social housing is not possible, steps should be taken to ensure that minipigs can interact with conspecifics by being able to see, smell, hear, and touch each other (e.g. snout contact). This can be achieved by housing minipigs in neighbouring pens separated by bars, transparent walls, and/or walls with holes.

Furthermore, extra measures should be taken to ensure a suitable enrichment program for single-housed animals, e.g. extra, positive staff interactions, or supervised physical interaction sessions with other minipigs.

Social behaviours are further enabled by letting the minipigs walk in the corridors of the barriers. This provides social enrichment, new stimuli, and potential for exercise, and is beneficial for all minipigs regardless of number of pen mates, but it is even more important with single-housed animals. Also, it has the added benefit of accustoming the minipigs to new surroundings which may improve mental resilience towards changes and events later in life.

Example of group-housed male Göttingen Minipigs.

Göttingen Minipigs regrouped in same sex group after weaning. The group will remain stable for as long as possible.

# Oral dosing of a gelatin formulation as an alternative to conventional oral gavage

*ABOUT STUDY INSIGHTS: Göttingen Minipigs are increasingly selected for all aspects of pharmaceutical research and are fully recognized as a reliable and established animal model by all regulatory authorities worldwide. This section aims at providing an insight into the wide use of Göttingen Minipigs within biological research.*

**Insight provided by:**
Tina Brønnum Pedersen, Christoffer G. Bavnhøj, Jette Bisgaard Boll, Henriette Hansen and Christian Skonberg | H. Lundbeck A/S, Denmark

With a special thanks to Haidar Jumaa for the initial investigations, and to animal and laboratory technicians at Lundbeck for skilled technical assistance.

*What is the study about?*
To stay compliant with Lundbeck's high standards of animal ethics and welfare, we wanted to explore the possibility of oral dosing of a gelatin formulation as an alternative to conventional oral gavage. The basic thought was to "gelatinize" the solution for oral gavage and investigate the feasibility of dosing this to minipigs by voluntarily ingestion. The study was planned and conducted successfully as a cross-functional effort.

*Why is it important?*
Oral gavage is a stressful procedure for pigs as the pig must be caught, fixated in order for the oral gavage tube to be placed, and restrained throughout the procedure. The oral gavage procedure may lead to physiological stress (Balcombe et al. 2004; Ellegaard et al. 2010) and the animals often become more aversive towards humans. It can also be a stressful procedure for the animal technicians as they must use force to restrain the animals, which in the long run lowers their motivation to do the work, with the risk of compassion fatigue. A typical oral gavage procedure requires at least three persons for fixating, placing the tube and dosing the animal. This procedure takes a few to five minutes.

From a practical point of view, the Danish work environment legislation does not allow heavy lifting (>12.5 kgs) (Dansk AT vejledning), which in practice limits the use of the minipigs for PO procedures for longer periods, as by approximately five months of age the minipigs usually weigh around 12.5 kg (Ellegaard minipig background data).

To improve animal welfare, improve working environment, and save resources, the usage of gelatin formulation was proposed as a new approach for oral administration.

*Study setup*
Five marketed therapeutic compounds were chosen as model compounds to compare the pharmacokinetics of dosing an oral solution via oral gavage and as an oral gelatin formulation. Selection of the compounds was based on their properties in terms of intestinal solubility and permeability, according to the Biopharmaceutics Classification System. The study was set up in a cross-over design in which the minipigs were given similar doses (mg/kg) of each of the compounds as both an oral solution and an edible gelatin formulation with a short wash-out period

| Phase | Treatment | Compound | Dose (mg/kg) |
|---|---|---|---|
| I | A, oral gavage | Paracetamol | 15 |
| | B, gelatin | | 15 |
| II | A, oral gavage | Hydrochlorothiazide | 1.5 |
| | B, gelatin | | 1.5 |
| III | A, oral gavage | Chlorothiazide | 8 |
| | B, gelatin | | 8 |
| IV | A, oral gavage | Griseofulvin | 12.5 |
| | B, gelatin | | 12.5 |
| V | A, oral gavage | Carbamazepine | 5 |
| | B, gelatin | | 5 |

**Table 1**
The cross-over study design for assessing peroral gavage vs. peroral gelatin formulation with listing of test compounds and doses.

between dosing. In total, the study was conducted over six weeks of dosing both gelatin and oral gavage every week (Table 1).

The gelatin formulations were prepared by gelatinizing the solution used for dosing via oral gavage. Due to solubility, one compound (griseofulvin) had to be formulated as a suspension with methylcellulose. The solutions and the suspension were mixed with a high-concentration (15% W/V) bovine gelatin solution that was liquified by melting (max 60 deg. C). Subsequently, the gelatin-containing mixtures were portioned out in 30 mL plastic-cups and solidified overnight at 5-10°C. A blue colorant was also added to the gels to facilitate visual inspection of the surroundings and the mouths of the minipigs for any gel formulation not swallowed (Figure 1). If some of the gel was not ingested, it was collected and weighed to correct the actual given dose.

*Training the pigs for dosing*

As the minipigs were not used to eating gelatin, they had to be trained to eat the gelatin with minimal chewing to ensure that they ate most – and preferably all - of the gelatin.

To reduce the time needed for training, the animals were ordered as habituated to human contact and touching at Ellegaard Göttingen Minipigs A/S. Furthermore, the least fearsome pigs, willing to interact with humans, were selected. This is something Ellegaard provide as a service.

When the animals arrived at our facility, the transport cages were situated at the entry of the pig pen and opened so the pig could enter the pen when ready. Feed was scattered on the floor in the pen as a teaser.

Socialization to our staff was initiated on the first day. Contact was attempted by going into the pen, kneeling, and waiting for the pig to come forward, but in such a way as to make sure the minipig did not feel cornered without an option to escape. However, as pigs are curious, they usually make contact either right away or within the first few days.

Working with positive reinforcement training, goodies were provided when the minipigs were doing as desired. It was important to select goodies that the minipigs liked and which most likely would not interfere with the study purpose. Also, the goodies should not provide too much energy, as the pigs might otherwise gain too much weight. We tried yoghurt, apples, and juice but after a bit of habituation, carrots seemed to best fulfil the criteria for goodies that the minipigs liked.

Thereafter, the positive reinforcement training was started by using a whistle to bridge a wanted behaviour to the carrot reward. When the pig had learned this, and was able to follow a target-stick, a vascular access surgery was performed to allow for easier repeated painless blood sampling from the minipig. This may also be achieved by a per-cutaneous catheter for single dosing. In our experience, under these circumstances the pigs are ready to participate in a study 21 days after arrival in our facility.


**Figure 1**
Three oral gelatin formulations evaluated for potential precipitation of compound on a light-box.

The pigs were trained to swallow the gelatin formulation before getting a piece of carrot. Current experience with training and dosing with the gelatin formulation was highly positive. Up to five gels could be administered within 10 min, and dosing and blood sampling was handled by one animal technician, in contrast to the at least three technicians required for oral gavage.

*Results from proof-of-Concept study*

Data from the comparison of dosing formulations to the minipigs, showed that when corrected for the actual doses (mg/kg), the two formulations performed similarly with regards to tmax, Cmax, and area-under-the-curve (AUC). One compound (griseofulvin) was dosed as a suspension at low concentration and here the two formulations also showed similar PK profiles (see Figure 2A and 2B for plasma profiles for paracetamol and griseofulvin, respectively).

*Which challenges have you met during the study?*

**Formulation aspects**

One challenge we experienced was regarding poorly soluble compounds, where it is often required to add solubility enhancing excipients to dissolve the entire dose. Not all solubility enhancing excipients were found to be compatible with the gelatin. One excipient, PEG400, was found to disrupt the integrity of the gelatin formulation and cause precipitate. Therefore, development of a gelatin formulation is not limited to identifying the most appropriate solubility enhancing excipient, but also to thoroughly assess the effect of excipients on the gelatin formulation, including precipitation of the drug compound and viscosity changes of the gelatin.

A further challenge was that the gelatin formulations had to be prepared a day in advance and thus the animal bodyweights had to be assessed based on last measured weight and growth curves. This required detailed communication between gel manufacturer and animal facility.

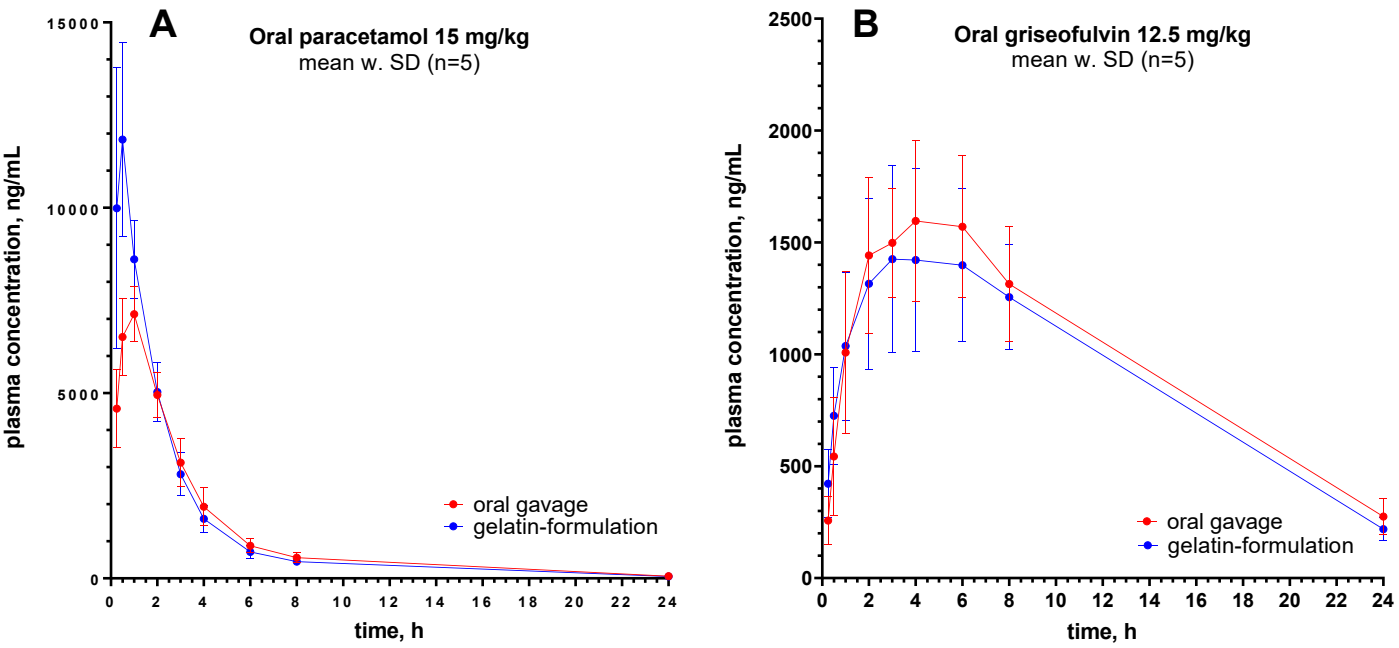


**Figure 2**
Plasma concentration profiles for paracetamol (A) and griseofulvin (B) following oral dosing as either gavage (red symbols) or with gelatin formulation (blue symbols).

In some instances, it was observed that not all the gelatin formulation was ingested by the pig, and any uneaten gelatin was collected and weighed to correct for the actual dose given. This entailed some extra requirements for documentation in the animal facility during dosing and appropriate corrections for actual doses when doing pharmacokinetic calculations.

**Minipig habituation and training**

Initially, the pigs were not willing to eat the gelatin, nor did they find it intersting. Several attempts were made to increase the curiosity of the pigs by providing frozen gelatin or adding artificial flavours (strawberry, caramel, and vanilla), but we eventually succeeded by adding shredded carrots into the gelatin. In the beginning, a lot of carrot was added, but for each training session the amount was decreased, and nothing was actually needed when fully trained.

Due to the large cross-over study conducted over 6 weeks, and the pigs not being allowed a weight of more than 12.5 kgs, we ordered the pigs quite young (4 kg ~1.5 months old). The younger minipigs were more apprehensive, compared to the older minipigs that we normally order (8 kg~4 months old), and the training had to be adapted with more time needed for socialization.

Due to the young age, the housing area was required to be warmer (28-30 °C). By coincidence, we found out that the increased room temperature caused the gelatin to melt after some hours. This could be one reason why at first the pigs did not seem interested in the gelatin, as the gelatin was provided on the floor, thinking that the pigs would eat it by themselves. But either the gel melted before they got to eat it, or they just played with the (melted) gel. We actually do not know, but if they start perceiving the gelatin as a toy, it seems difficult to re-train them to eat it if they first consider the gel as enrichment.

Whether it is the younger age or the method for habituation to the gel, that made the pigs eating the gelatin more troublesome than expected in the beginning, is yet to be elucidated.

*What is next step?*

It is the plan to proceed with investigating the feasibility of using gelatin formulations as an alternative to oral gavage. It seems necessary to get more experience with training the minipigs and continuously refine the positive reinforcement training. Especially, best practices for training pigs to eating the gelatin is important to explore further.

Furthermore, investigating the pharmaceutical limitations of the gelatin formulation is essential. Our next step is to enhance the knowledge of formulating test compounds with different physico-chemical properties, and to explore excipients which may be used to aid in these formulations. For instance, stabilization of a suspension in a gelatin formulation may be challenged by lack of


**Figure 3**
Compound dispersed as solid particles (non-dissolved) in a gelatin matrix. The illustration demonstrates the sedimentation of compound at the bottom, i.e., as an inhomogeneous suspension.

homogeneity. If compound particles sediment at the bottom, it hinders dose-adjustment to the animal bodyweight by dividing the gelatin formulation ( Figure 3).

Application of gelatin formulation mainly seems relevant for PK studies and single dose studies. The gelatin formulation is currently not considered relevant for toxicology studies as during these studies, with higher doses and sometimes longer duration, the pigs will expectedly experience adverse effects of the test compounds and therefore may not feel as hungry or curious as normal, which can affect the willingness of the pigs to eat the gelatin.

*Any learnings you would like to share?*
We found that it paid off to be creative if the pigs did not like what was presented to them. As described above, adding carrots to the gel seemed to be a solution to train the pigs to eat the gelatin. Also, in the beginning, the gel was cut into smaller pieces and presented, and over time the gelatin pieces could be made increasingly larger.

The gelatin for training purposes may be manufactured in larger batches and kept in the freezer to prolong durability and shape. However, if drugs or compounds are added to the gelatin, stability analysis must be made to ensure the compound is not affected by the freezing and thawing processes.

## REFERENCES

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2 Balcombe, Jonathan P.; Barnard, Neal D.; Sandusky, Chad. Laboratory Routines Cause Animal Stress. Journal of the American Association for Laboratory Animal Science, Volume 43, Number 6, November 2004, pp. 42-51(10) ingentaconnect.com/content/aalas/jaalas/2004/00000043/00000006/art00009
3 Danish Working Environment Authority, WEA guideline D3.1 Lifting, Pulling and Pushing - Arbejdstilsynet (at.dk) at.dk/en/regulations/guidelines/lifting-pushing-and-pulling-d-3-1/ (In English) / at.dk/regler/at-vejledninger/loeft-traek-skub-d-3-1/ (In Danish)
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5 Lars Ellegaard, Andrew Cunningham, Sandra Edwards, Nanna Grand, Timo Nevalainen, Mark Prescott, Teun Schuurman, and under the auspices of the Steering Group of the RETHINK Project. Welfare of the minipig with special reference to use in regulatory toxicology studies. Journal of Pharmacological and Toxicological Methods 62 (2010) 167–183

# Spotlights

## Join the Pain in Research Group

Animal research may involve painful procedures yet little is known or shared on pain relief and pain scoring of pigs. The mission of this group is to change the current situation by knowledge sharing on pain and analgesia in pigs.

The aim of the group is to:

- Create a community of people working with pigs and minipigs who are interested in pain and pain management
- Improve animal welfare according to the 5Rs (Reduction, Replacement, Refinement, Responsibility and Relevance)
- Highlight this subject by creating free accessibility to scientific material and webinars as well as practical experience
- Establish a group to foster collaborations in this field e.g. standardization of pain scoring

The group will adress:

- Which drugs to use and when, treatment regimens, side effects
- Non-pharmacological methods for reducing pain and suffering
- PK-PD questions
- Pain scoring and pain biomarkers
- Refinement of pain models

Interested in joining the group? Scan the QR-code to know more.


*Publication*

## "Genomic evidence for the suitability of Göttingen Minipigs with a rare seizure phenotype as a model for human epilepsy"

Epilepsy affects roughly 2% of people worldwide, yet current treatments only reduce seizure frequency and severity without modifying the disease. The search for effective drugs is limited by the inadequacy of existing animal models to fully represent the disease. A rare subset of Göttingen Minipigs (GMPs), having shown spontaneous epileptic convulsions, might serve as a better model for studying epilepsy. This study genetically analyzed affected GMPs and used fibroblast cultures to examine genetic variants affecting gene expression. Several newly identified genes related to calcium metabolism and transcription factors regulating genes previously linked to human epilepsy-type disorers suggest this rare subset of GMPs could be a more accurate model for epilepsy research, indicating a need for further validation.

Read the open access paper: DOI 10.1007/s10048-024-00750-2

## Biosecurity during transportation of minipigs

A vital part of maintaining the high health status of Göttingen Minipigs is to have strict biosecurity measures in all aspects of housing, husbandry, facility management, and logistics- including transportation of minipigs. To ensure full control of the transport of the animals, Ellegaard Göttingen Minipigs has its own vehicles and chauffeurs. For long-distance deliveries a dedicated, experienced subcontractor is employed. The vehicles are equipped with climate control, and all necessities (feed, water, etc) are brought from the facility. Our chauffeurs wear dedicated coats, gloves, safety footwear, hair nets, and masks during handling and regular oversights. To limit the risk of contamination, our chauffeurs do not enter your facility when they arrive but deliver the minipigs to the loading bay entrance.

Our customers are responsible for return of emptied and rinsed transport boxes to the chauffeurs for subsequent disinfection. This is an essential part of biosecurity to reduce the risk of transfer of microbiological agents from the delivery site.

To maintain the barrier status at the premises of Ellegaard Göttingen Minipigs, biosecurity processes are also in place for empty vehicles returning after delivery of the minipigs. The cars arrive at the Transportation Unit, separate from the rest of the facility. The unit contains a car wash and washing area for transportation boxes. After washing and disinfection, the vehicles and boxes are moved to a clean garage and ready for a new delivery.

Thank you for your continued support in upholding the high health status - at our facility and yours.



## "Visit me where I was born"

Since Copenhagen is the host city of this year's EUROTOX, Ellegaard Göttingen Minipigs has decided to invite toxicologists from around the world to visit their facilities. The scientific programme includes presentations on:

- Introduction to our company and Göttingen Minipigs as a large animal model
- **Tour of the facility, incl. a visit to the viewing loft to see the Göttingen Minipigs**
- Göttingen Minipigs in toxicology
- Spontaneous histopathological findings in Göttingen Minipigs in toxicity studies
- The future of Göttingen Minipigs

Lunch and transportation between the venue hotel and Ellegaard Göttingen Minipigs are included in the programme.

Add the visit when you are making your programme bookings during your EUROTOX registration on www.eurotox2024.com.

# 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

## Up-coming courses

### 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

**Extract of course description:** Learn how to properly approach, lift, and carry minipigs and try out different restraint and dosing techniques incl. oral dosing and blood sampling from a sling. Depending on experience, we will also cover endotracheal intubation, catheterisation methods for vascular access and humane euthanasia. Limited seats.

*Course: Handling & Dosing*

*Course: Handling & Dosing*

### 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

**Extract of course description:** This course focuses on the physical demands of handling animals. It emphasizes ergonomic principles for comfortable work, highlighting that comfort matters more than ergonomics alone. The course offers practical tips and interactive sessions to enhance well-being and prevent work-related injuries in animal care, relevant to all involved in laboratory animal care.

### 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

**Extract of course description:** In this course, you will learn the theory of different training methods, how this will improve your understanding and communication with Göttingen Minipigs, and how this can benefit your studies. The course includes theoretical lectures and practical training with clickers. Learn what can be achieved with good and planned training. Limited seats.

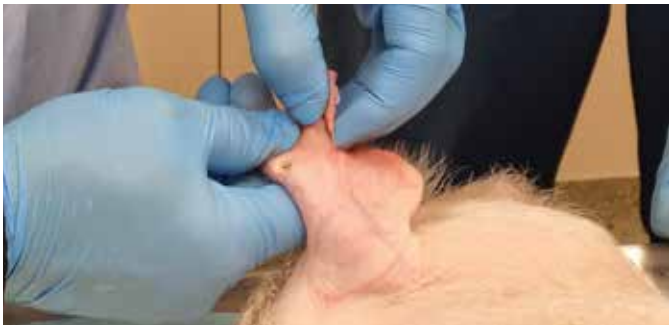
## On-demand

### 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



*Course: Vascular Access*

### 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*

NEWS FROM

# Ellegaard Göttingen Minipigs A/S

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

## ESG profile

Our responsibility towards prioritising and incorporating sustainable and energy-saving solutions in our operations is no secret, and we are happy to share our initiatives, which include:

- Solar panels
- Geothermal heat solution
- Straw-fired boiler (as support to the geothermal heat)
- Charging stations for electric cars
- Planting of climate forest

Find more information about our ESG initiatives on our website: minipigs.dk/about-us/todays-company

Solar panels and 2 out of 8 charging stations for electric cars.

1 out of 808 newly planted trees in a climate forest

## Appointments and anniversaries

1 April 2024 Carina Anker celebrated her 10th anniversary with us.

Carina is our Animal Welfare Technician dedicated to continuously evaluating and improving the welfare of our Göttingen Minipigs.

5 April 2024 Pernille Birch celebrated her 20th anniversary with us.

Pernille is the Barrier Manager of our Research Barrier, where our research facility is located and where we breed IgG Humanized Göttingen Minipigs and Göttingen Micropigs.

6 April 2024 Kenneth Ib Jensen celebrated his 25th anniversasry with us.

Kenneth is part of our general management team and ensures that our outdoor areas are neat, so that guests and employees can enjoy the settings upon arrival and during breaks.

Welcome to Ida Sofie Vilhelmsen as new Animal Caretaker.

Ida has joined forces with our team of Animal Caretakers at one of our breeding barriers to ensure that our Göttingen Minipigs thrive and have everything they need.

## Where to meet us in 2024

| CONFERENCE | DATE | LOCATION |
|---|---|---|
| Scand-LAS | 21-24 May | Tampere, Finland |
| Minipig Research Forum | 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 |
| STP-I | TBD | TBD |
| LASACON | TBD | TBD |

# Göttingen Minipigs Symposium

## June 11th 2024

*Cambridge, MA*

9 am - 5 pm
Lunch and networking cocktail hour included.

***For further information contact:***

**Ellegaard Bioresearch**
+1 410-693-7101 | jca@minipigs.com

**Marshall BioResources**
+1 315 587 2295 | infous@marshallbio.com

## PROGRAM

**FDA** | Renqin Duan
Regulatory acceptability of the minipig as an alternative non-rodent specie in drug development

**University of Antwerpen** | Steven Van Crutchen
Species selection in drug safety testing: minipigs as alternative to NHPs

**Charles River Lab** | Laure Penard
CRO Innovation and Quality in use of the minipig for nonclinical pharmaceutical development

**J&J/Janssen Pharmaceutical** | Jeffrey Fernandez
The Use of Göttingen Minipigs in Pharmaceutical Research

**Gubra** | Heidi Lindgreen Holmberg
Expanding translational knowledge on PK/PD using minipigs in obesity drug discovery

**Marshall Bioresources** | Michelle Salerno
Dosing/care and handling minipigs

**Exemplar Genetics** | Chris Rogers
Genetically modified minipigs: Expediting the path to the clinic

**Ellegaard Göttingen Minipigs** | Andres Eskjær Jensen
The development of new transgenic strains of minipigs (IgG Humanized and Micropig)

*Click or scan to register*

# 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.*

Felgendreff P, Lawrence JM, Hosseiniasl SM, et al.
**Clinical characterization of a hypersensitivity mixed bacterial and fungal dermatitis in a translational model of porcine NASH**
Published: Jan 2024
DOI: 10.3389/fcimb.2023.1277045

Bergamo ETP, de Oliveira PGFP, Campos TMB, et al.
**Osseointegration of implant surfaces in metabolic syndrome and type-2 diabetes mellitus**
Published: February 2024
DOI: 10.1002/jbm.b.35382

Stærk K, Langhorn L, Halle B, Andersen TE
**Urinary bladder catheterisation of female pigs: Influence of bladder content and Escherichia coli urinary tract infection on procedural outcome**
Published: February 2024
DOI: 10.1177/00236772231169344

Pernold CPS, Lagumdzic E, Stalder M, et al.
**Species comparison: human and minipig PBMC reactivity under the influence of immunomodulating compounds *in vitro***
Published: January 2024
DOI: 10.3389/fimmu.2023.1327776

Hammer SE, Duckova T, Gociman M, et al.
**Comparative analysis of swine leukocyte antigen gene diversity in Göttingen Minipigs**
Published: February 2024
DOI: 10.3389/fimmu.2024.1360022

Najafi P, Reimer C, Gilthorpe JD, et al.
**Genomic evidence for the suitability of Göttingen Minipigs with a rare seizure phenotype as a model for human epilepsy**
Published: April 2024
DOI: 10.1007/s10048-024-00750-2

Lützhøft DO, Bækkgård C, Wimborne E, et al.
**High fat diet is associated with gut microbiota dysbiosis and decreased gut microbial derived metabolites related to metabolic health in young Göttingen Minipigs**
Published: March 2024
DOI: 10.1371/journal.pone.0298602

Shen J, Silva RLE, Zhang M, et al.
**Suprachoroidal gene transfer with nonviral nanoparticles in large animal eyes**
Published: March 2024
DOI: 10.1126/sciadv.adl3576

Koopmans SJ, Binnendijk G, Ledoux A, et al.
***Momordica charantia* fruit reduces plasma fructosamine whereas stems and leaves increase plasma insulin in adult mildly diabetic obese Göttingen Minipigs**
Published: March 2024
DOI: 10.1371/journal.pone.0298163

Bernardini C, Nesci S, La Mantia D, et al.
**Isolation and characterization of mammary epithelial cells derived from Göttingen Minipigs: A comparative study versus hybrid pig cells from the IMI-ConcePTION Project**
Published: March 2024 (E-pub)
DOI: 10.1016/j.rvsc.2024.105244

Langston JL, Myers TM
**Development and characterization of an automated behavioral assessment platform for the Göttingen minipig**
Published: April 2024
DOI: 10.1016/j.toxlet.2024.02.009

Swart DH, de Haan M, Stevens J, et al.
**Safety, tolerability and toxicokinetics of the novel mitochondrial drug SUL-138 administered orally to rat and minipig**
Published: March 2024
DOI: 10.1016/j.toxrep.2024.03.009

Liu H, Inoue R, Koyanagi M, et al.
**Comparison of the fecal bacterial microbiota in mice, rats, and pigs after oral administration of alpha-glycosyl isoquercitrin**
Published: 2024
DOI: 10.2131/jts.49.151

Bergamo ETP, Witek L, Ramalho IS, et al.
**Sustained Release of Salicylic Acid for Halting Peri-Implantitis Progression in Healthy and Hyperglycemic Systemic Conditions: A Gottingen Minipig Model**
Published: May 2024
DOI: 10.1021/acsbiomaterials.4c00161

Horseman T, Rittase WB, Slaven JE, et al.
**Ferroptosis, Inflammation, and Microbiome Alterations in the Intestine in the Göttingen Minipig Model of Hematopoietic-Acute Radiation Syndrome**
Published: April 2024
DOI: 10.3390/ijms25084535

Poirier B, Pasquier O, Chenede X, et al.
**R2R01: A long-acting single-chain peptide agonist of RXFP1 for renal and cardiovascular diseases**
Published: March 2024
DOI: 10.1111/bph.16338

Zinno C, Agnesi F, D'Alesio G, et al.
**Implementation of an epicardial implantable MEMS sensor for continuous and real-time postoperative assessment of left ventricular activity in adult minipigs over a short- and long-term period**
Published: April 2024
DOI: 10.1063/5.0169207

Bøgh N, Sørensen CB, Alstrup AKO, et al.
**Mice and minipigs with compromised expression of the Alzheimer's disease gene *SORL1* show cerebral metabolic disturbances on hyperpolarized [1-$^{13}$C]pyruvate and sodium MRI**
Published: March 2024
DOI: 10.1093/braincomms/fcae114

Rode F, Bundgaard C, Areberg J, et al.
**Stress-free blood sampling in minipigs: A novel method for assessing 24-h cortisol profiles and drug effects on diurnal and ultradian rhythms**
Published: April 2024
DOI: 10.1016/j.vascn.2024.107504

Berger O, Choi W, Ko CH, et al.
**Long-Circulating Vasoactive 1,18-Octadecanedioic Acid–Terlipressin Conjugate**
Published: April 2024
DOI: 10.1021/acsptsci.3c00305

Barone F, Bunea I, Creel K, et al.
**An Automated Visual Psychophysics Method to Measure Visual Function in Swine Preclinical Animal Model**
Published: March 2024
DOI: 10.1167/tvst.13.3.8

Changelian P, Xu C, Mnich S, et al.
**ATI-1777, a Topical Jak1/3 Inhibitor, May Benefit Atopic Dermatitis without Systemic Drug Exposure: Results from Preclinical Development and Phase 2a Randomized Control Study ATI-1777-AD-201**
Published: March 2024
DOI: 10.1016/j.xjidi.2023.100251

Iacono D, Hatch K, Murphy EK, et al.
**Proteomic changes in the hippocampus of large mammals after total-body low dose radiation**
Published: March 2024
DOI: 10.1371/journal.pone.0296903



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