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# Recent updates in the use of Göttingen Minipigs as a relevant model in drug development

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## RECENT UPDATES

- DART: shortened design for EFD-type studies
- Juvenile Animal Studies: Pre-weaning cardiorespiratory monitoring

## RECENT UPDATES

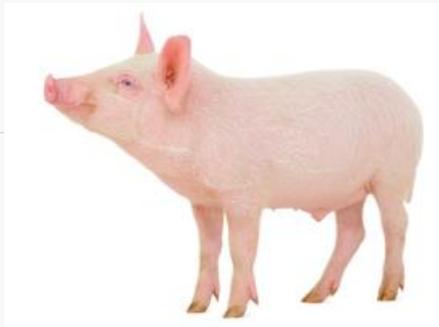
- DART: shortened design for EFD-type studies

# The laboratory bred Göttingen Minipig

## We all know the Göttingen but perhaps a little less for EFD studies

### Characteristics and Advantages

- Susceptibility to human teratogens
- **Supply of time-mated females**
- Early sexual maturity: 4-7 months
- Large litter size: 5-6 fetuses/litter
- Short organogenesis: GD11 to GD35
- Available historical control data
- **Regulatory acceptance**



Reproductive Toxicology 64 (2016) 162–168

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Reproductive Toxicology

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The incidence of congenital malformations and variations in Göttingen minipigs

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**ABSTRACT**

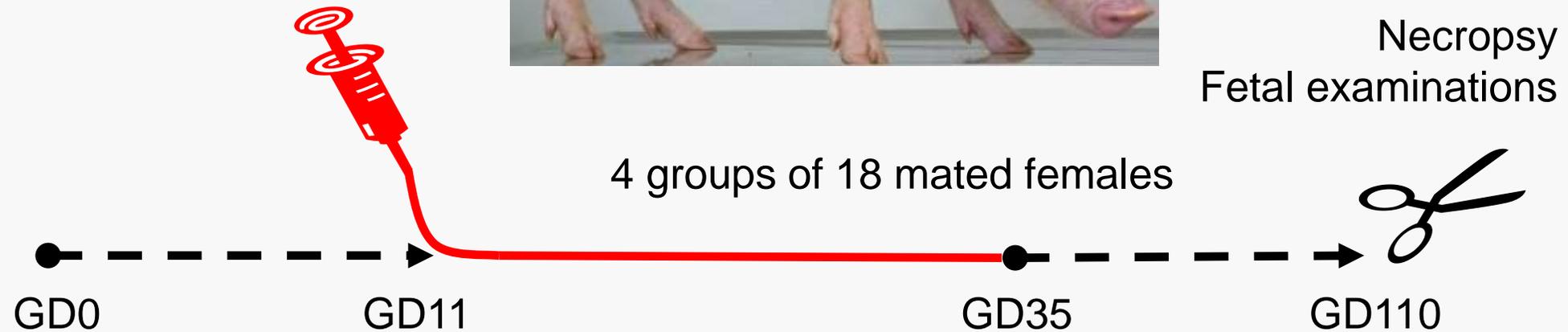
Knowledge of the incidence of spontaneous congenital abnormalities is critical for the accurate interpretation of findings in teratogenicity studies in any species. In this paper, results of the examination of 1739 neonatal Göttingen Minipigs are presented. Over the 2-year period under consideration, the incidence of external and visceral malformations was less than 0.2 and 0.1%, respectively. The most common external malformations were syndactyly, limb hyperflexion, domed head and scoliosis. The most common internal malformations were undescended testes, ventricular septal defect, diaphragmatic hernia and atrial septal defects. Pentadactyly and variation in the aortic arch's bifurcation (absent truncus bicaroticus) were the most common variations. These data will help support the use of the Göttingen Minipig as a non-rodent species in embryofetal development studies where concerns persist about the availability of background data.

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# ICH EFD STUDY

## Minipig Embryo-Fetal Development (EFD) study – traditional design

- GLP compliance
- Toxicokinetics



In-life phase: 3 months, 3 weeks and 3 days!

# ALTERNATIVE APPROACH FOR MINIPIG EFD STUDIES

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- **Question (2008!):** do we need to wait until the end of gestation in the minipig to detect chemical effects on embryo-fetal development?



# ALTERNATIVE APPROACH FOR MINIPIG EFD STUDIES

- Early termination described in ICH S5 (R3) for NHP EFD studies:
  - C-sections at GD100 with natural birth not until around GD160
  - **“Stage when adequate morphological examinations can be performed”**
- Mid-term C-sections (~GD60) with external fetal examinations already performed for minipig DRF studies as standard
- By mid-term, organogenesis (dosing period) and one third of fetal period completed
- **→ Why not go a step further with full fetal examinations?**

# DETECTION OF TERATOGENIC EFFECT AT GD60

- Test material selected : **pyrimethamine**
  - Know human and swine teratogen
  - Previously evaluated in the minipig with high incidence of birth defects without maternal toxicity (Hayama *et al*, 1985)
- **Validation studies:**
  - Time-mated Göttingen females provided by Ellegaard (arrival by GD5)
  - Dosing from GD11 to GD35 with pyrimethamine by oral gavage
  - C-sections on or close to GD60
  - Fetal examinations:
    - External
    - Visceral (fresh body and fixed head)
    - Skeletal

# DETECTION OF TERATOGENIC EFFECT AT GD60

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- **Results:**
  - No maternal toxicity
  - Severe decrease embryo-fetal survival
  - Malformations detected (consistent with those described for piglets):
    - Cleft palate
    - Micrognathia
    - Ectrodactyly
    - Malformed limbs/hooves
    - Open eyes

# Skeletal Examinations

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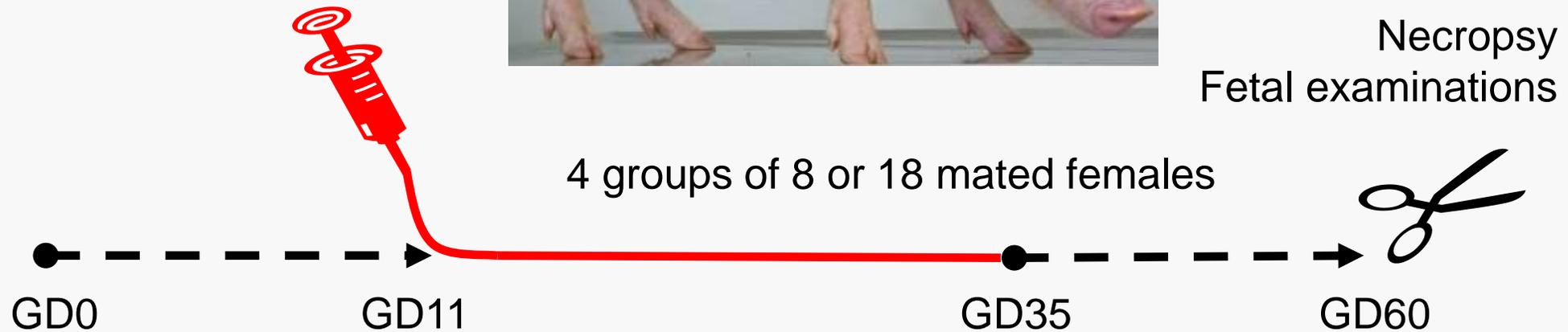
- Easier processing of the fetuses at GD60 vs GD110
- Lower fetal weight (53 g at GD60 vs 329 g at GD110)
- Minipig fetal size at GD60 comparable with rabbit at GD29



# ICH EFD OR pEFD STUDY

## Minipig EFD or pEFD study – shortened design

- GLP compliance
- Toxicokinetics



# ADVANTAGES OF ALTERNATIVE APPROACH

- **Study comparison** for the minipig (GD60 and GD110) and rabbit (GD29)

Approximate duration	Minipig GD110 (32 pEFD or 72 dams)	Minipig GD60 (32 pEFD or 72 dams)	Rabbit GD29 (88 dams)
In-life	18 or 20 weeks (incl. 2 or 4 w. staggered start)	11 or 13 weeks (incl. 2 or 4 w. staggered start)	5 weeks
Fetal skeletal processing	7 weeks	3 weeks	3 weeks
Fetal skeletal examinations	2 or 3 weeks	1 week	2 weeks
<b>Total study duration</b>	<b>27 or 30 weeks</b>	<b>15 or 17 weeks</b>	<b>10 weeks</b>

# DETAILS PUBLISHED IN 2018

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## A shortened study design for embryo-fetal development studies in the minipig

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### ARTICLE INFO

#### Keywords:

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Mid-term fetal examinations  
Pyrimethamine  
Skeleton double staining

### ABSTRACT

The minipig is accepted from scientific and regulatory perspectives for the safety evaluation of drug candidates on embryo-fetal development. The relative size and the duration of gestation (112–115 days) in the minipig is, however, considered a drawback compared with routine smaller species. We evaluated if study duration and cost could be optimized without impacting scientific validity by performing all terminal procedures around mid-gestation (60 days). At this stage, minipig fetal size is not too dissimilar to full term rabbit and therefore better suited to fetal processing/examination compared with at the end of gestation. Despite encountering higher than anticipated embryo-fetal death, morphological defects clearly associated with a known teratogen, pyrimethamine, were detected. Although the gonads are poorly differentiated macroscopically at mid-term, a histological examination confirmed that external sexing of the fetuses was accurate. Double staining of the bone and cartilage of the mid-term fetal skeleton allowed a more refined examination.

# Regulatory Advice in 2021

- So, Ten +++ Years After!
- We haven't "Changed the World" but we have made a small change in developmental toxicology
- Proposed shortened design submitted to and discussed with both US and EU regulatory authorities
- First GLP regulatory study with shortened design is currently ongoing at the CRL-Lyon facility

TEN YEARS AFTER

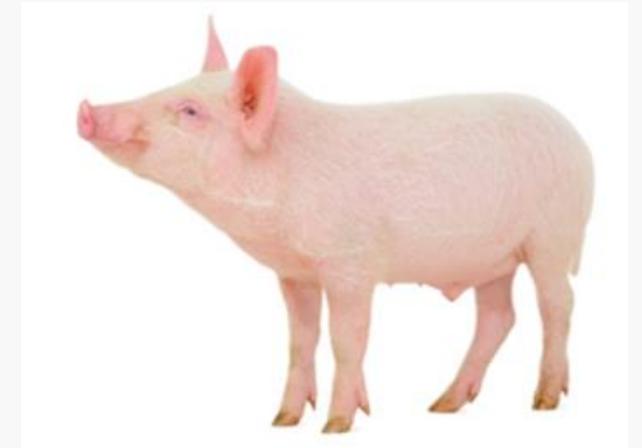


## RECENT UPDATES

- Juvenile Animal Studies: Pre-weaning cardiorespiratory monitoring

# NON-RODENT JUVENILE STUDIES

- The rat is the most widely used species for juvenile toxicity studies
- However, non-rodent species are required punctually
  - Pediatric only indication – no adult data from healthy volunteers or patients
  - Rat unsuitable model with identified concerns
  - Scientific justification (pharmacology) for a non-rodent species
- The Göttingen Minipig is cited in the ICH S11 guideline and some of the advantages and disadvantages of the species are described



# MINIPIG NEONATAL PIGLET SELECTION CRITERIA

## ADVANTAGEOUS ATTRIBUTES

- We can pre-select piglets shortly after birth based on weight and physical/functional development parameters:
  - Selected piglets: **standing with open eyes, suckling and walking**
  - “Doubtful” piglets: « runts » less than 300 g (fetus weight!) not meeting one of the above criteria (open eyes or walking)
  - Eliminated piglets: weak pups that cannot stand/move without stimulation with closed eyes



# CONSIDERATIONS FOR STUDY DESIGN

## Need for a range of scientific and technical expertise

- Class of drug
- Development of specific organ system(s)
  - **Cardiovascular**
  - Central Nervous System
  - Gastrointestinal
  - **Pulmonary**
  - Immune
  - Renal
  - Reproductive
  - Skeletal (growth)



- **Cardiorespiratory assessments may need to be included in non-rodent juvenile animal studies performed to support the development of pediatric pharmaceutical candidates**

# ETISENSE AND DECRO SYSTEM

- **Etisense: Start-up localized in France (Lyon)**
- <https://www.etisense.com/>
- **DECRO system:**
  - Bluetooth jacket for cardiac and respiratory monitoring of small animals, without surgery:
    - Respiratory function (respiratory frequency, tidal and minute volume)
    - Cardiac function: ECG, heart rate
    - General Activity / Movement
  - Acquisition system and Software
  - Already available for rat but “small animals” also includes juvenile non-rodent species!



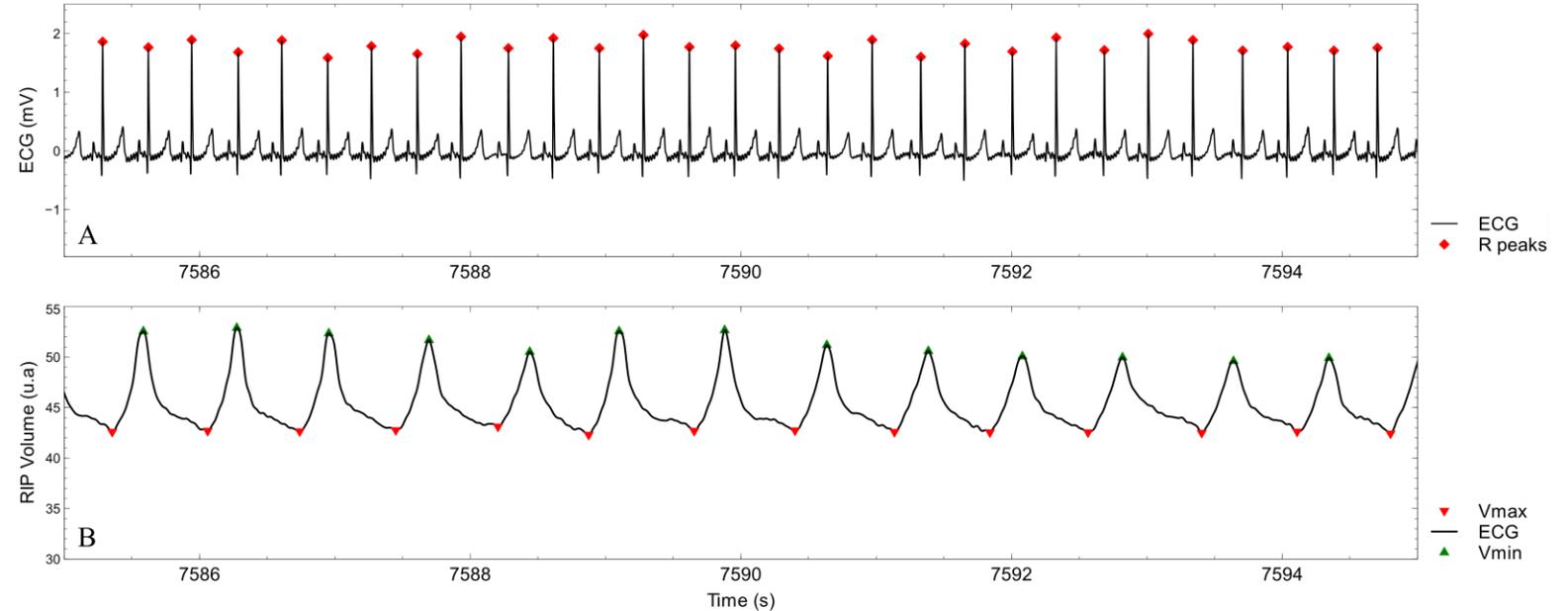
# DECRO SYSTEM FOR JUVENILE MINIPIGS

## Collaborative Validation study between Etisense and CRL

- Once weekly evaluations from 1 to 4 weeks of age
- Three biosignals recorded simultaneously for up to 20h after dosing with Baclofen
  - ECG intervals
  - Respiration by inductance plethysmography (RIP bands): frequency, tidal and minute volume
  - General activity by accelerometry

# RESULTS

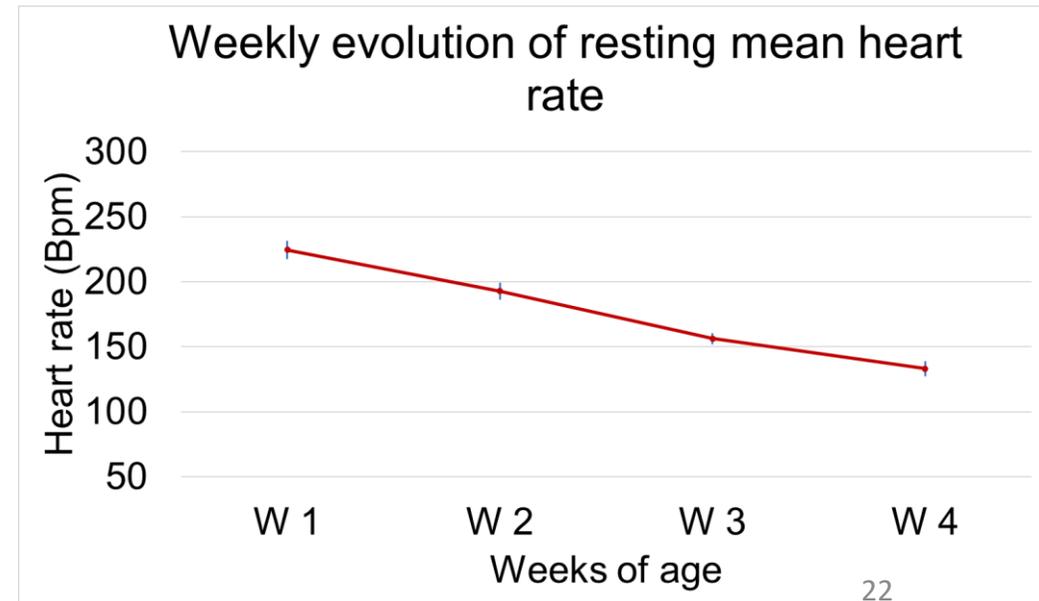
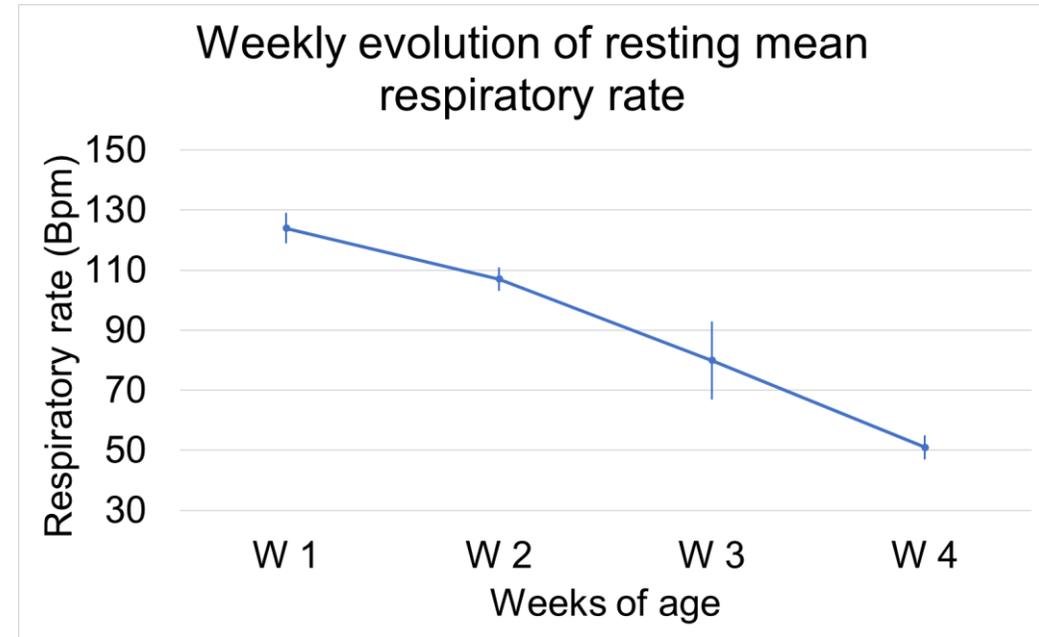
- Simultaneous non-invasive recording of quality electrocardiographic, respiratory and activity signals were possible
- The jacket had no impact on growth and behavior through to weaning
- Overall, 80-90% of ECG and 40% of RIP signals (after exclusion of impaired signals due to activity artifacts) were analyzable



ECG waves and respiratory Inductive Plethysmography signals in the first week of life

# RESULTS

- Over the first four weeks of life, respiratory and heart rates decreased with age
- Cardiorespiratory parameters obtained using the Decro<sup>®</sup> jacket were comparable with results obtained from restrained animals in previous studies (snapshot ECG and visual assessment of respiratory rate)
- Recording for up to 20 hours was made possible by improving both the robustness and comfort of the jacket and recording system



# POSTER PRESENTED AT BDRP MEETING 2021

### Results

#### Cardiorespiratory and activity analysis :

- Overall, 80-90% of ECG (Figure 2A) and 40% of RIP signals (after exclusion of impaired signals due to activity artifacts – Figure 2B) are analyzable.
- Over the first four weeks of life, respiratory and heart rates decreased with age (Figure 3 and Figure 4).

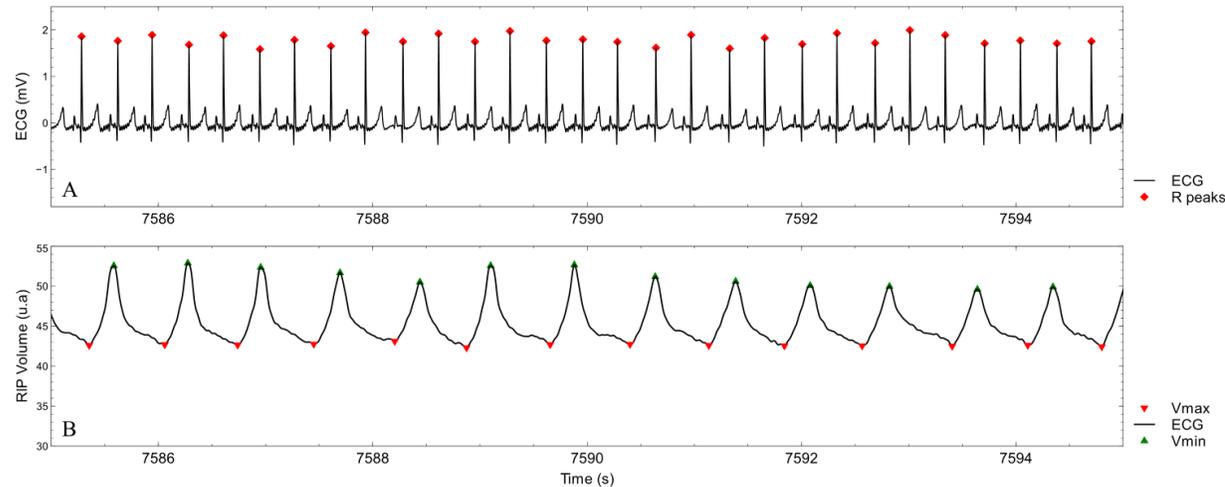


Figure 2: (A) ECG waves in the first week of life, recorded at rest. (B) Respiratory Inductive Plethysmography signals at rest with clear respiratory cycles

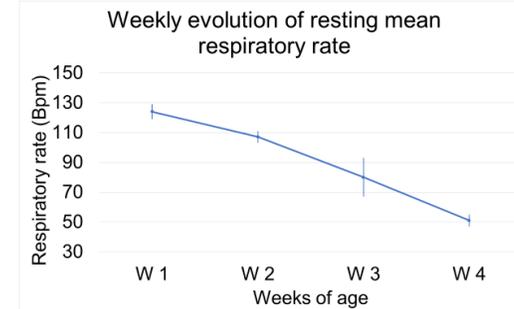


Figure 3: Weekly evolution of resting mean respiratory rate in Phase 1

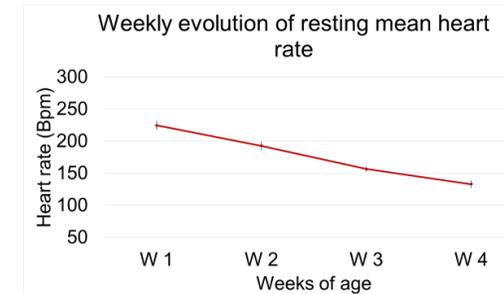


Figure 4: Weekly evolution of resting mean heart rate in Phase 1

### Conclusion

In conclusion, simultaneous non-invasive recording of quality electrocardiographic, respiratory and activity signals and subsequent analyses were achieved in the juvenile Göttingen Minipig using the jacketed Decro® system from the first week of life (up to weaning). Fitting juvenile animals with this device at different post-natal ages had no impact on their growth and behavior. Cardiorespiratory parameters obtained using the Decro® jacket were comparable with results obtained from restrained animals in previous studies (snapshot ECG and visual assessment of respiratory rate). Recording for up to 20 hours was made possible by improving both the robustness and comfort of the jacket and recording system.

# Thank You!

**Shortened EFD study**

**Céline Pique, Paul Quesada, Audrey Blondel and  
Lars Friis Mikkelsen**

**Juvenile minipig cardiorespiratory monitoring**

**Laure Penard, Timothy Flenet, Charles Eynard, Corinne Simon,  
Christophe Bory and Stéphane Baudet**