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# Transdermal Measurement of Glomerular Filtration Rate in Mechanically Ventilated Piglets

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## Abstract

Transdermal measurement of glomerular filtration rate (GFR) has been used to evaluate kidney function in conscious animals. This technique is well established in rodents to study acute kidney injury and chronic kidney disease. However, GFR measurement using the transdermal system has not been validated in pigs, a species with a similar renal system to humans. Hence, we investigated the effect of sepsis on transdermal GFR in anesthetized and mechanically ventilated neonatal pigs. Polymicrobial sepsis was induced by cecal ligation and puncture (CLP). The transdermal GFR measurement system consisting of a miniaturized fluorescence sensor was attached to the pig's shaved skin to determine the clearance of fluorescein-isothiocyanate (FITC) conjugated sinistrin, an intravenously injected GFR tracer. Our results show that at 12 h post-CLP, serum creatinine increased with a decrease in GFR. This study demonstrates, for the first time, the utility of the transdermal GFR approach in determining renal function in mechanically ventilated, neonatal pigs.

## Introduction

A practical and quantitative evaluation of renal function is the glomerular filtration rate (GFR) measurement, which tells how well the kidneys filter blood based on the clearance principle[1]. An earlier method of measuring GFR entails the intravenous injection of exogenous compounds such as inulin or sinistrin, conducting serial measurements of plasma/urinary levels to detect their clearance[2,3]. This method is cumbersome, requiring the serial collection of plasma and urine samples[4]. An alternative is the measurement of endogenous metabolic end-products such as creatinine. However, this is time-consuming and, at times, inaccurate, as it is not only filtered by the glomerulus but also secreted by the tubules[5,6]. Furthermore, creatinine level is influenced by gender, age, diet, and muscle mass[7,8,9].

A more precise, minimally invasive, and widely used measure of GFR is the use of transdermal GFR monitors, which measure real-time GFR in animals[4,10]. Sinistrin, a highly soluble and freely filtered exogenous renal marker, is labeled with fluorescein-isothiocyanate

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None.

(FITC). This conjugated compound is injected intravenously, and real-time kidney function can be assessed without collecting blood and urine samples[11]. The use of transdermal GFR measurement has been validated in rodents[12], dogs[13], and cats[14], but not in swine.

Porcine species share several anatomical and physiological characteristics with humans, making them ideal animals for studying various human diseases[15]. The use of pigs in translational biomedical research has become increasingly popular and preferred over rodent models because it mimics human physiology and pathophysiology[16]. Neonatal pigs are of interest in understanding the mechanisms of diseases unique to pediatric patients[17]. Moreover, the recent advancement in pig to human organ transplantation puts an urge to expand the diagnostic tools for preclinical and clinical trials[18,19,20,21]. This paper, for the first time, provides a guide for the use of the transdermal device in measuring GFR in neonatal pigs.

## Protocol

The procedures are written according to national standards for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Tennessee Health Science Center (UTHSC).

NOTE: Piglets in the experimental group are subjected to cecal ligation and puncture, while the sham group only undergoes opening of the abdomen without cecal ligation or puncture. Piglets in both groups are kept under anesthesia for 12 h post-procedure to allow enough time for sepsis and acute kidney injury (AKI) to ensue in the experimental group. Transdermal GFR measurement will ensue at 8 h post-procedure for a total of 12 h.

### 1. Piglet supply and housing

1. Identify a local hog farm that can provide neonatal piglets aged 3–5 days. Schedule the delivery early in the week to complete the experimentation before any piglets are older than 7 days.

   NOTE: The supplier provided three to five piglets on Mondays for this experiment; by Friday, the piglets would have undergone the experiment. Using the same sex and near similar age is essential to avoid confounding factors.

2. Upon the piglet's arrival, ensure they have an individual identification (e.g., an ear tag and a record that includes weight and age).

3. House the piglets in a lab animal care unit (LACU) under the care of a licensed veterinarian. The animals are housed as a group in a spacious pen with a solid concrete floor that is easily washed with water to maintain good sanitation.

4. Add a piece of furniture such as a heavy ball to allow for environmental enrichment and stimulation.

5. Ensure that the LACU provides optimal environmental conditions, including the following key elements: sanitation, nutrition, temperature control, ventilation, and day-night cycle by controlling illumination.

6. Have the veterinarian check on the piglet daily, including weight measurement, to inform the investigator if any piglet appears sick, which may necessitate exclusion from the experiment.

7. Leave the piglets for at least 1 day to acclimatize to the environment, which helps minimize the stress.

## 2. Pre-operative preparation

1. Prepare the surgical station before initiating the experiment. This includes a heating pad, catheters, a ventilator, an endotracheal tube, heparinized saline, and a bag of ringer lactate fluid.

   NOTE: Piglets have poor thermoregulatory capacitance and are prone to hypothermia which alters hemodynamics[22,23]. Therefore, allowing enough time for the heating pad to warm up is essential.

2. Prepare 10 mg/mL of α-chloralose by mixing it with saline at 60 °C until the mixture is clear. Do not overheat the solution to avoid crystallizing of the medication upon cooling. Filter with a syringe filter (size 0.22 μm) before administering to the piglets.

3. Draw up anesthetic medication based on animal weight-Ketamine: 20 mg/kg and Xylazine: 2.2 mg/kg. Use α - chloralose (5 mL/kg) to maintain anesthesia.

## 3. Anesthesia

1. Perform induction of anesthesia in the pig pen, an environment familiar for piglets, to avoid undue stress.

2. Gently pick the piglet by the back legs and administer Ketamine: 20 mg/kg and Xylazine: 2.2 mg/kg into the rear leg at the semimembranosus/semitendinosus muscle, using a 23 G ¾ needle.

3. Allow a few minutes for the medications to take effect. Check for the adequate anesthesia level by ensuring that the animal is relaxed enough to be immobile, with loss of palpebral reflex and jaw tone to allow ease and safe transportation to the surgical station. Assess the palpebral reflex by touching the inner corner of the eye; absence of blinking indicates adequate anesthesia.

## 4. Tracheostomy

NOTE: This experiment is non-survival, so a tracheotomy is performed to establish an airway for mechanical ventilation. Tracheostomy is a quick and easy procedure, as opposed to endotracheal intubation, which is challenging in piglets given their head and upper airway anatomy[24,25]. Additionally, laryngospasm is commonly reported during intubation, resulting in a prolonged period of hypoxia and hypercarbia that may compromise results[26].

1. Position the piglet in dorsal recumbency. Identify the cricothyroid cartilage by palpating the prominence of the thyroid cartilage which feels firm. Sterilize the area using povidone-iodine and 70% ethanol before applying a sterile drape.

2. Using a a surgical blade, make a 2–3 cm ventral midline incision inferior to the caudal end of the thyroid cartilage.

3. Using a curved mosquito hemostat, bluntly dissect the overlying subcutaneous tissues and muscles (sternohyoideus and cutaneous coli) until the cricothyroid membrane and the first few tracheal rings are visualized. When dissecting, be cautious to avoid injuring any blood vessels.

4. Obtain a clear view of the cricothyroid membrane and tracheal rings[24], then use a pair of long mixter right angle forceps to elevate the structures.

    1. With a pair of small scissors, make a small cut at the cricothyroid membrane or the first tracheal ring. Extend the cut horizontally to ~0.5 cm to pass a 3.0 mm endotracheal tube.

    2. Insert the tube to the 5 cm mark. Ensure bilateral chest expansion and breath sounds prior to securing the tube.

5. Pass umbilical tape around the trachea to secure it in place. Additional tape is used to secure the tube to the base of the jaw.

6. Switch on the ventilator, connect the endotracheal tube, and roll the specific knobs (eg. SIMV knobs, PEEP knobs, etc) to select the following baseline settings. Pressure Control Mode: **synchronized intermittent mechanical ventilation** (SIMV); **peak inspiratory pressure** (PIP) - 15; **positive end-expiratory pressure** (PEEP) - 5; **Rate**- 20; **I-time** - 0.6. Following the first blood gas analysis, adjust the ventilator settings according to the blood gas results, with the goal of maintaining adequate oxygenation and ventilation.

## 5. Femoral vessel cannulation

1. Establish the airway and ventilation, before switching attention to the femoral vessels for venous access and invasive blood pressure monitoring. The femoral artery is identified by feeling a pulse at the groove between the sartorius and gracilis muscles, and the vein can be found just medial to the artery.

2. While the piglet is lying in a dorsal recumbent position, sterilize the groin area using povidone-iodine and ethanol, and apply an appropriately sized drape.

3. Use a surgical blade to create a 3–4 cm longitudinal incision, starting cranially at the inguinal crease and extending distally along the femoral canal.

4. Apply blunt and sharp dissection, using mosquito curved forceps and scissors, respectively, to dissect down to the level of the femoral neurovascular bundle. The bundle can be found deep in the body of the gracillis muscle[27]. Circumferentially dissect the femoral artery and vein over the course of 2–3 cm to allow for cannulation. Ligate small side branches if necessary.

5. Apply a 3.0 silk tie at both the artery and vein’s proximal and distal ends to apply traction. Tie the distal silk suture on both the vein and artery, ligating the vessels.

6. Beginning with the femoral vein, maintain distal and proximal traction on the silk ties and then use a pair of micro scissors to create a venotomy.

7. Next, use a vein pick catheter introducer to open the vessel while inserting a pre-measured polyurethane catheter with an internal diameter × outer diameter of 0.86 mm × 1.32 mm. Once inserted, tie the proximal 3.0 silk suture to fixate the catheter. Flush the catheter with 3 mL of heparinized saline solution (1 U/mL). This solution can be made by adding 0.5 mL of heparin to 50 mL of normal saline.

8. Insert an invasive blood pressure catheter using the same approach above to create an arteriotomy and pass the catheter.

   NOTE: Maintaining distal and proximal traction is essential to minimize blood loss when accessing the artery.

9. Once the catheters are secured, cover the site with saline-soaked gauze, and if necessary, the skin may be sutured using a 3.0 silk suture to prevent infection.

## 6. Maintenance of anesthesia, fluid and blood gas

1. Once venous access is secured, inject a loading dose of 50 mg/kg of α-chloralose intravenously. Monitor the level of anesthesia throughout the experiment, using jaw tone and palpebral reflex, and administer additional boluses of α-chloralose at a dose of 20 mg/kg, as needed, to maintain the animal under deep anesthesia.

2. Infuse ringer lactate at a rate of 4 mL/kg/h throughout the experiment as maintenance fluid. For example, if the piglet weight is 3 kg, then the fluid infusion rate is 12 mL/h.

3. For bedside gas analysis, draw an arterial blood sample in a heparinized blood gas syringe and present the sample to the analyzer machine. Select the option **arterial blood gas**, and wait for ~2–3 s for the the analyzer to present the blood-draw needle.

   1. Carefully insert the needle into the end of the syringe containing the blood sample. Wait for the analyzer to aspirate the required sample and withdraw the syringe. Allow the machine to analyze the blood gas and present the results.

   2. Based on the results, adjust the ventilator to maintain the pH between 7.35--7.45, partial pressure of carbon dioxide (PCO2) between 35–45 mmHg, and partial pressure of oxygen (PaO2) between 80–150 mmHg. The settings differ based on the ventilator type, but largely involves increasing or reducing the respiratory rate using appropriate knobs to compensate for hypoxia and/or hypercapnia.

4. Draw 3 mL of blood into a light green tube (Lithium Heparin). Centrifuge the sample at 2000 x*g* for 15 min, maintained at 4 °C to extract plasma. Once completed, the plasma can be analysed immediately for serum creatinine level with the bedside chemistry analyzer or stored at −80 °C for later analysis.

5. Monitor the temperature hourly using a rectal probe thermometer and adjust the heating pad temperature to maintain piglet temperature between 101 to 103 °F.

## 7. Experiment group; cecal ligation and perforation (CLP)[25,28,29]

NOTE: For piglets in the experiment group, perform CLP to induce polymicrobial sepsis[28] and monitor the animal for 12 h post-surgery to allow enough time for severe sepsis to ensue. Transdermal GFR recording starts at 8 h post-cecal ligation to allow for 4 h of recording.

1. Use a surgical blade to create a 5–6 cm left paramedian vertical incision, as the cecum in pigs lies in the left paralumbar fossa[30]. Dissect down the abdominal wall layers, avoiding injury to the superficial epigastric vessels.
2. Once the peritoneal layer is incised, use a retractor to improve access to intrabdominal structures.
3. Identify the spiral colon in the upper left quadrant of the abdomen. Trace the spiral colon, caudally and dorsally, to locate the cecum. The ileum is seen joining the spiral colon at the base of the cecum.
4. Ligate the cecum just distal to the ileocecal junction (Figure 1).
5. Using a 18 G needle, make seven punctures in the cecum and extrude feces into the peritoneal area.
6. Close the abdomen in layers with a 3.0 silk suture using either simple interrupted or continuous stitches. A stapler may also be used to close the skin layer if available.

## 8. Sham group

1. Follow the steps 7.2–7.4 as above. After identifying the cecum, place it back untouched and close the abdominal wall similarly.
2. Monitor piglets in the sham group for 12 h to eliminate any confounding bias attributed to prolonged exposure to anesthesia.

## 9. Transdermal GFR device setup

1. After 8 h of cecal ligation, get ready to initiate transdermal measurement of GFR.
2. Use the MB service software version 3.0 to adjust the sampling rate on the GFR device. Briefly, connect the transdermal GFR device to the computer software using the USB connector. Open the software, click **connect**, and adjust the timing to 4000 ms. Click **write** to save the settings.

   NOTE: This gives up to 6 h of total sampling time. In the pigs, transdermal GFR is completed in 4 h. For experiments that require sampling up to 12 h, choose the 8000 ms option.

3. Attach the dual-sided adhesive patches with a clear window to the device. Attach the device to one side, ensuring the light-emitting diode overlies the clear window to allow tracer detection.

4. Shave the area overlying the lateral thoracic wall. Attach the battery to the device and immediately stick the adhesive patch with the device in place and make sure it is well secured (Figure 2). Since the piglets are deeply anesthetized, tape might be unnecessary to hold the device in place.

   NOTE: The adhesive patch alone is enough to secure. However, in procedures where the animal would be manipulated, become active, or where anesthesia might be disrupted, it might be important to apply a tape. A bandage might also be an alternative approach[31].

5. A baseline recording of 3–5 min is required before administering FITC-sinistrin.

## 10. FITC-sinistrin preparation and injection

1. Prepare a mixture of FITC-sinistrin with saline solution to a final concentration of 50 mg/mL. The dose administered to the piglet is 20 mg/kg. FITC-sinistrin is supplied in powder form.

   NOTE: The FITC-sinistrin may also be administered through a peripheral venous catheter inserted in the auricular vein. It is essential to achieve a high peak level by administering FITC-sinistrin as a push bolus through the femoral vein venous catheter.

2. Attach the syringe with medication to one side of a three-way stop cock and a saline flush on the other side of the stop cock. Push the FITC-sinistrin and immediately follow with a 5 mL saline bolus before closing the three-way stop cock to the piglet vein.

## 11. Transdermal GFR recording

1. Keep the device attached to the piglet for 4 h. During this time, keep the piglet under anesthesia using intermittent doses of α-chloralose at a concentration of 20 mg/kg to avoid any motion artifact.

2. At the end of the 4 h, remove the device and immediately disconnect the battery.

## 12. GFR measurement

1. Connect the transdermal GFR device to the computer using the USB connector provided by the supplier.

2. Open the reading software to retrieve data from the device. Save the raw data by clicking the sequence: **connect**, **read**, **re-name**, and **save**. As instructed in the manual, process and evaluate the saved data in the analysis software.

3. Briefly, open the software ver. 3.0 and import the data. Adjust the offset, start, and end positions using the automated markers. Remove artifacts if necessary,

and click **fit**. This gives a readout that shows FITC-sinistrin clearance in minutes (t1/2). The t1/2 is subsequently used to calculate the tGFR[32,33] as below:

$$\text{tGFR[mL/min/100 g BW]} = \frac{20[\text{mL/100 g BW}]}{\text{t1/2(FITC-sinistrin)[min]}}$$

NOTE: In consultation with the manufacturer, the conversion factor used for pigs is 20 (indicating that 20% of the body weight is extracellular space), as opposed to 21.33 in rats (tGFR in mL/min) and 14,616.8 in mice (tGFR in μL/min). This is because GFR is accurately measured as a function of extracellular fluid[34,35], which in turn is dependent on body weight[36].

### 13. Piglet euthanasia

1. Collect 3 mL of blood after 12 h of CLP for further biochemical analysis.
2. Euthanize the piglet by administering 0.2 mL/kg of pre-mixed mixture of 20% sodium pentobarbital and Phenytoin Sodium intravenously.
3. Harvest the right kidney for histopathologic study before taking the piglet to the morgue.

## Representative Results

In this section, we present for the first time, the representative data from the use of transdermal GFR in neonatal pigs. We used a cecal ligation and puncture model which has previously been shown to decrease kidney function[28]. Accordingly, we hypothesized that in our CLP pigs, there should be an acute drop in GFR corresponding to AKI, and this should be detected on the transdermal GFR device as increased clearance time ($t_{1/2}$), thereby validating its use in pigs. Seven male piglets were included, three sham and four sepsis. The two groups had comparable weights (Figure 3A). As expected[28], 12 h sepsis increased serum levels of C-reactive protein (CRP), a bacteremia and sepsis marker (Figure 3B). Representative FITC-sinistrin clearance curves in sham vs septic piglets are shown (Figure 4 A,B), with AKI shown by overlaying the sham and sepsis curves (Figure 4C). AKI is shown by an increased area under the curve for the CLP pigs. This can be visibly seen when the sham curve is layed on the CLP curve. The average half-life for FITC-sinistrin in the sham and sepsis groups were 114 and 537 minutes, respectively (Figure 5A). The average GFR in the sham group was 5.1 mL/min/100 gm of the body weight, while in the sepsis group, it was 1.06 mL/min/100 gm of the body weight (Figure 5B). An additional animal was excluded as the probe was displaced, which disturbed the clearance curve and time. Whereas 12 h serum creatinine (a biomarker of acute kidney injury) did not change in the sham group, it was increased from ~ 0.6 to 1.08 mg/dL in the septic pigs (Figure 6).

## Discussion

This paper describes practical steps to determining kidney function in pigs using the miniaturized transdermal GFR monitors and FITC-sinistrin in a mechanically ventilated,

anesthetized neonatal pig model. Previous papers have established experimental transdermal GFR protocols in rodents[11,12,14], but no protocols exist in pigs.

Recently, there has been a drive to explore alternative animal models to solve intractable diseases and ease the burden of kidney disease in humans. Unfortunately, many of these approaches have had translational limitations due to size, anatomical, and physiological differences. Rodents' renal anatomy and pathophysiology have major differences when compared to humans[37]. Since the human and pig systems share similar anatomical and functional characteristics, the porcine model may be a more realistic pathophysiological model of human diseases[38,39]. Pigs are now widely used to delineate pathophysiology and in drug development. With the publication of the pig genome, alongside successful transgenic production of disease-specific models, the porcine model stands to take a more critical role in translational research[40,41].

Inulin clearance remains the most accepted means of GFR determination, but is impractical in large animal models due to the need for continuous infusion of inulin, catheterization of the bladder, and its time-consuming and cumbersome nature[42]. Serum creatinine and blood urea nitrogen (BUN) are commonly used to measure renal function in preclinical studies, but because creatinine is secreted in the tubules and urea is increasingly reabsorbed in dehydration, these markers have proved to be poor in estimating renal function[5,43]. Crucially, tubular creatinine secretion was found to cause overestimation of GFR when used as a marker of renal function in the pigs[6]. Also, due to their body habitus, a rise in creatinine is more likely to be seen in large animal models when compared to rodents. A study in mice revealed a 1.5-fold rise in serum creatinine 6 h post-cecal ligation[44]. Previously, we showed a rise in creatinine in neonatal pigs at 6 h post-CLP[28]. In this study, we kept the animals for a longer duration, ~12 hours post-cecal ligation to allow enough time for significant AKI and a subsequent rise in creatinine. As in our previous study, we confirmed the induction of sepsis by a rise in serum levels of CRP, an inflammation and sepsis marker. In this study, and as previous papers show, the severity of sepsis following CLP is dependent on the length of ligation and number of punctures[44].

A protocol to measure GFR in pigs using Iohexol has previously been validated in pigs[37], but in contrast, the transdermal GFR procedure is a marked improvement. It is less cumbersome, avoids repeated blood or urine sampling, and offers a real-time window into renal function and the possibility of repeated, serial measurements in the same animal[45]. This study provides practical guidelines for transdermal GFR determination in pigs.

As established by other groups, the most critical steps are the correct fixation of the device to the animal and the bolus injection of FITC-sinistrin. The measuring device must be well fixed to the skin surface to prevent movement artifacts on the trace. Because pigs are less hairy than rodents, using a depilatory cream is not required. A clean shave with a clipper might be all that is needed. This minimizes the depilation associated increase in the half-life of FITC-sinistrin, whose mechanism is unknown[12]. For proper fixation, a double-sided adhesive patch and tape are required to hold the device in place. The optimal device placement locations are the lateral thoracic wall and ventral abdominal region. These areas correlated with fewer movement artifacts.

When injecting the FITC-sinistrin, the correct and entire dose must be injected in one fluid motion into the vein. When the injection is interrupted and restarted, it creates multiple "mini-peaks" on the clearance curve. The tail vein is routinely used for small rodents, but the auricular ear vein offers a more accessible and prominent route in the pigs. A cannula can be placed in the ear vein for multiple measurements in conscious pigs. An important distinction to note in the sampling time is that, as opposed to rodents (~1–2 h), pigs lasts longer (~4 h), which approximates the time it takes for FITC-sinistrin to be cleared from the circulation. To the best of our knowledge, this is the first paper detailing transdermal GFR via FITC-sinistrin clearance in pigs. So, no citations exist for reference. The measuring time used ~4h was arrived at, via consultations with the manufacturer. This sampling time is comparable to a prior study validating transdermal GFR in other non-rodent mammals[14].

In evaluating transdermal GFR in piglets, there are a few factors that must be considered. One-compartment models are known to overestimate GFR significantly[46]; we use the three-compartment kinetic model which is more accurate, providing three-way communication of the intravenously injected marker between the plasma, extracellular space, and deeper components[46]. Also, these are mechanically ventilated piglets under very deep anesthesia for ~12 h. Since anesthesia influences renal function[47,48], it might be worth taking that into account in procedures that require long sedation or where experimental maneuvers require additional anesthesia alongside GFR monitoring. Finally, and perhaps most crucially, neonatal piglets have still-developing renal systems with immature nephrons that function at a fraction of the adult animal[49]. Hence, they demonstrate lower GFR and renal function[50].

As previously indicated, transdermal GFR in pigs is not an absolute measure of sinistrin concentrations in the blood. Its only an estimation of decay in fluorescence over time[12]. The use of a conversion factor attempts to mitigate this, by expressing GFR in mL/min. However, because the conversion factor is dependent on extracellular space, which in turn relies on body weight[34,35,36], it is possible for wide variations to exist if weight is not controlled for, or if the extracellular space is not accurately defined[51,52].

Additionally, skin pigmentation appears to affect transdermal FITC-sinistrin clearance[12,31]. In our studies, we found that the pigmented pigs showed decreased signal. In one instance, we did not detect signal in an intensely dark colored pig. However, since background signal tends to be reduced in pigmented animals[12], we found that GFR values were largely comparable. One solution to this is to opt for lighter colored areas of the skin when placing the device. Since these pigs were largely used in a surgical model of disease, with several forms of lighting and heat sources involved, one must account for potential movement artifacts on the GFR traces *via* reflected light absorbed from surrounding skin[12]. One solution to this might be to minimize infrared light during recording or covering the devices in foil.

In summary, this study offers a simple and reliable method for measuring glomerular filtration rate in neonatal pigs using the transdermal measurement of FITC-sinistrin clearance. Moreover, our data supports the utility of the system in evaluating kidney function in the settings of acute kidney injury.

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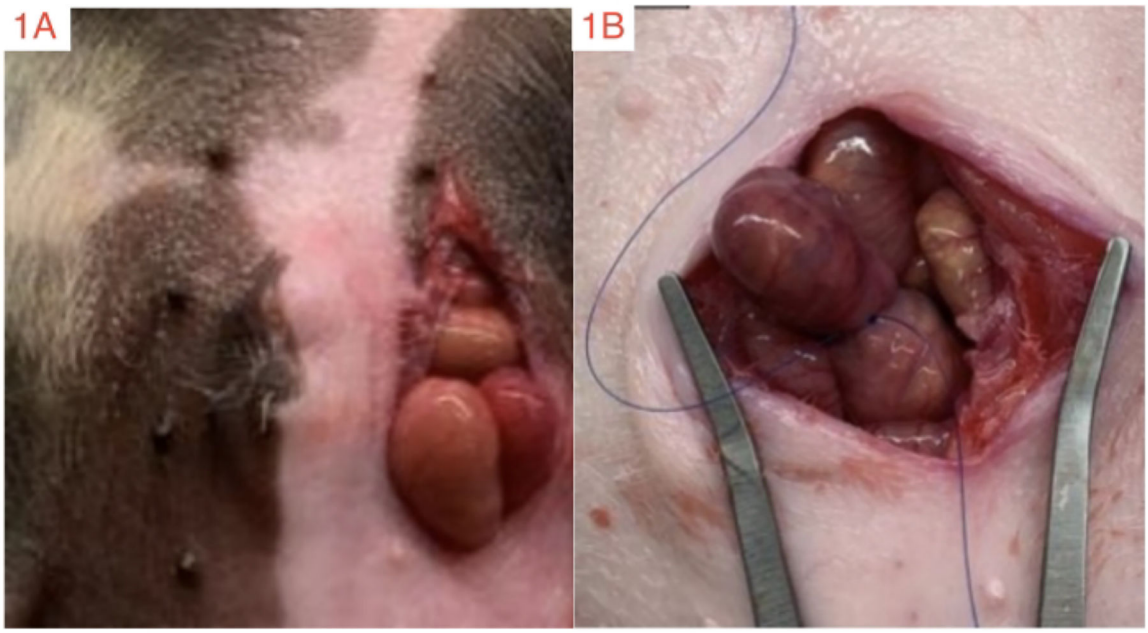


**Figure 1: Cecum ligation surgery.**
**(A)** Cecum identified and brought to the exterior. **(B)** Cecum ligated at the base with a silk tie before puncturing with a needle.

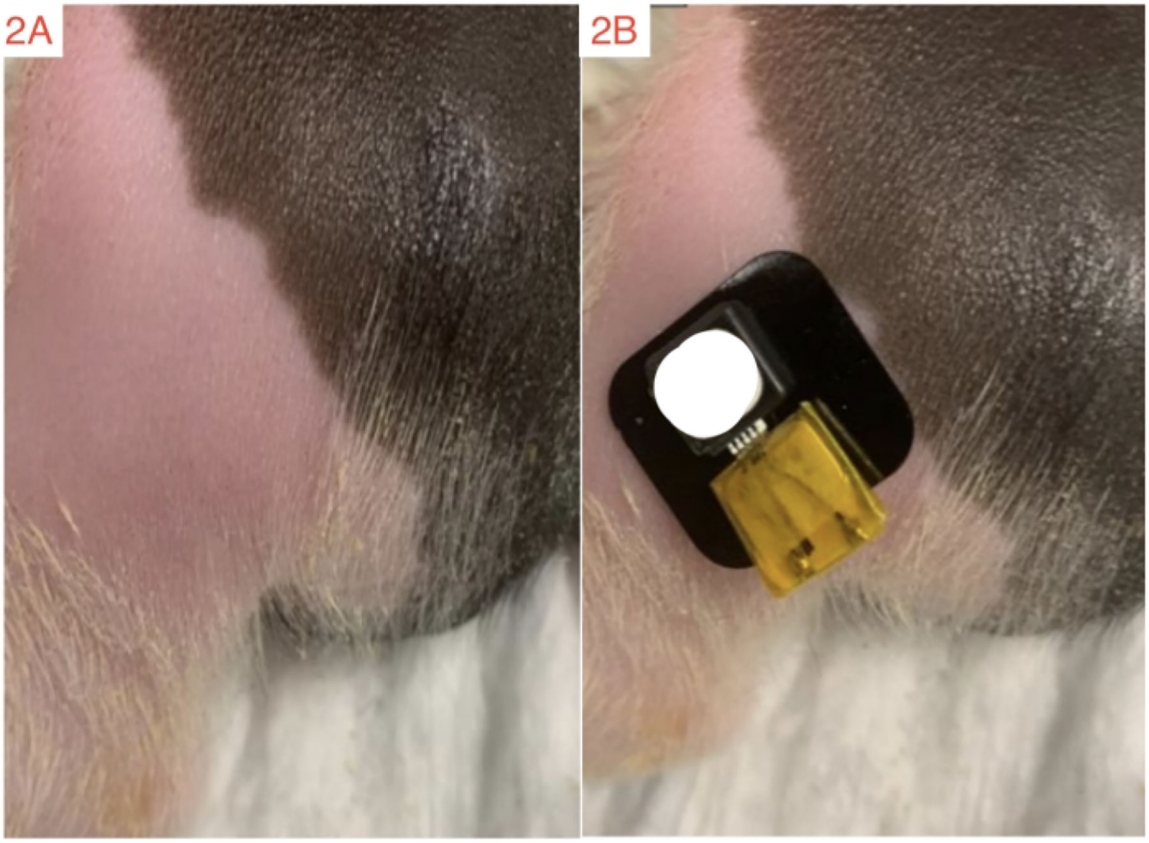


**Figure 2: Attachment of transdermal device to the skin.**
**(A)** Skin shaved prior to attachment of adhesive patch. **(B)** Transdermal GFR device attached to the adhesive patch.

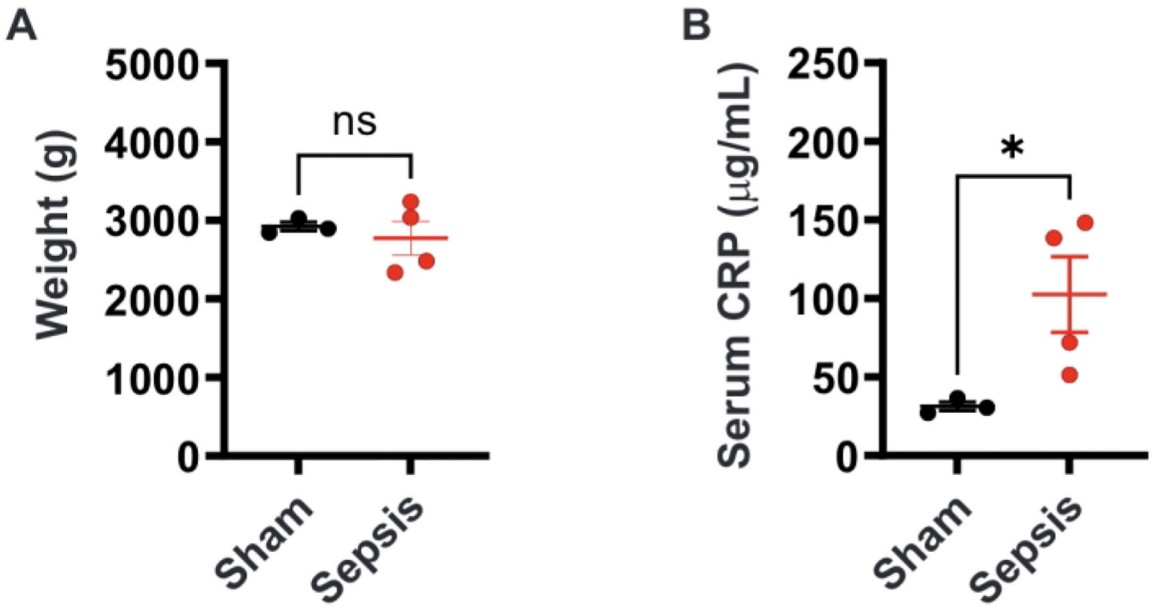


**Figure 3: Representative results.**

**(A)** Weight of the piglets used in this study and **(B)** Serum C-reactive protein (CRP) levels in mechanically ventilated sham and septic male piglets (unpaired t-test).

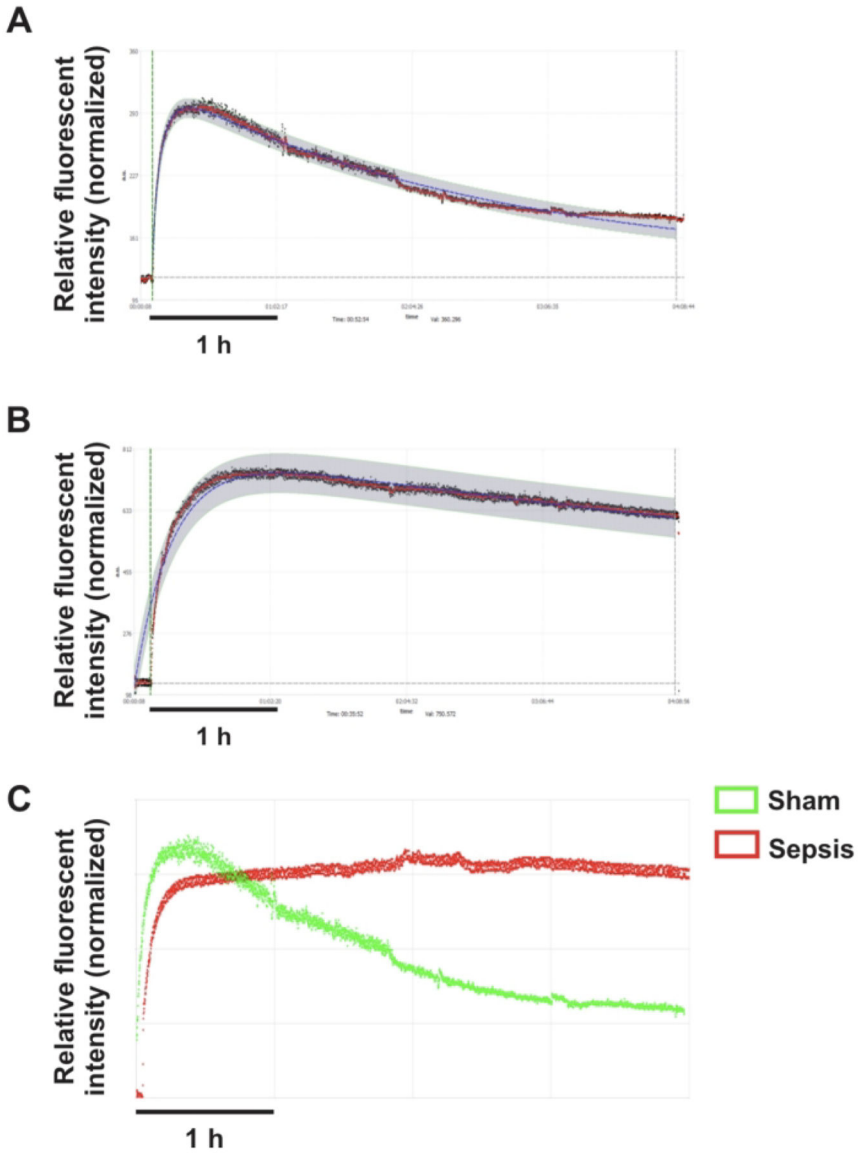


**Figure 4: Representative FITC-sinistrin clearance curves in mechanically ventilated sham and septic male piglets.**

**(A)** 12 h sham, **(B)** 12 h sepsis. Septic pigs present with impaired renal function as demonstrated by an increased area under the curve. Black data points represent raw data, blue lines the three-compartment fit, green lines the 95% confidence intervals, and red line the filtered data. **(C)** Overlay of representative curves to reflect the degree of divergence from baseline in septic pigs. The sepsis curve (red) showed minimal clearance of FITC-sinistrin, indicating AKI.

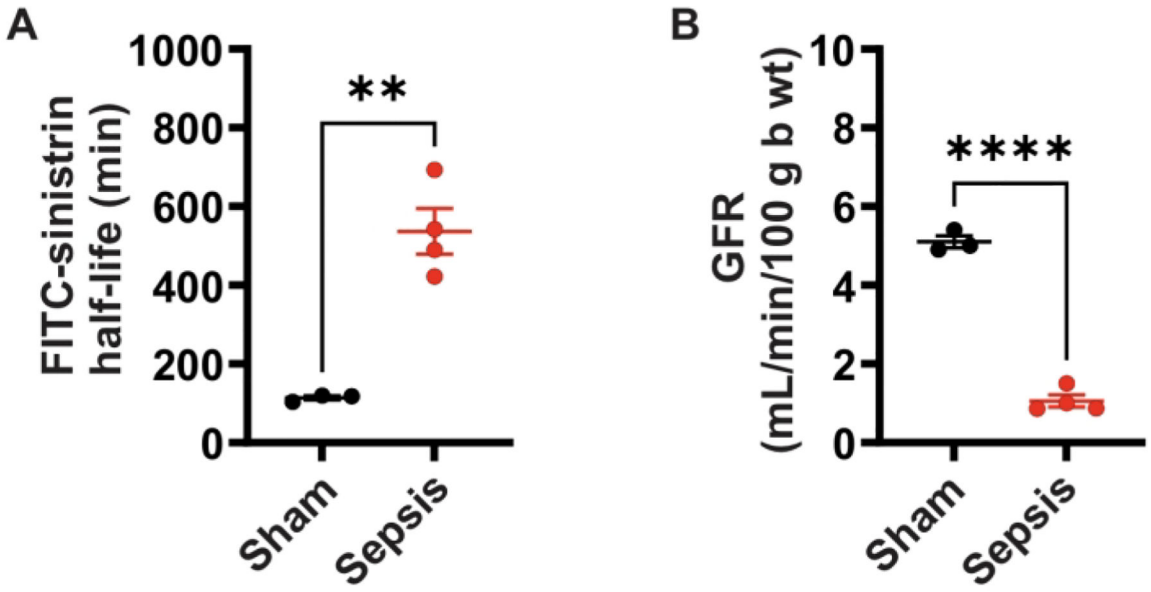


**Figure 5: Representative results.**

**(A)** FITC-sinistrin half-life and **(B)** GFR plots in mechanically ventilated sham and septic male piglets (unpaired t-test).

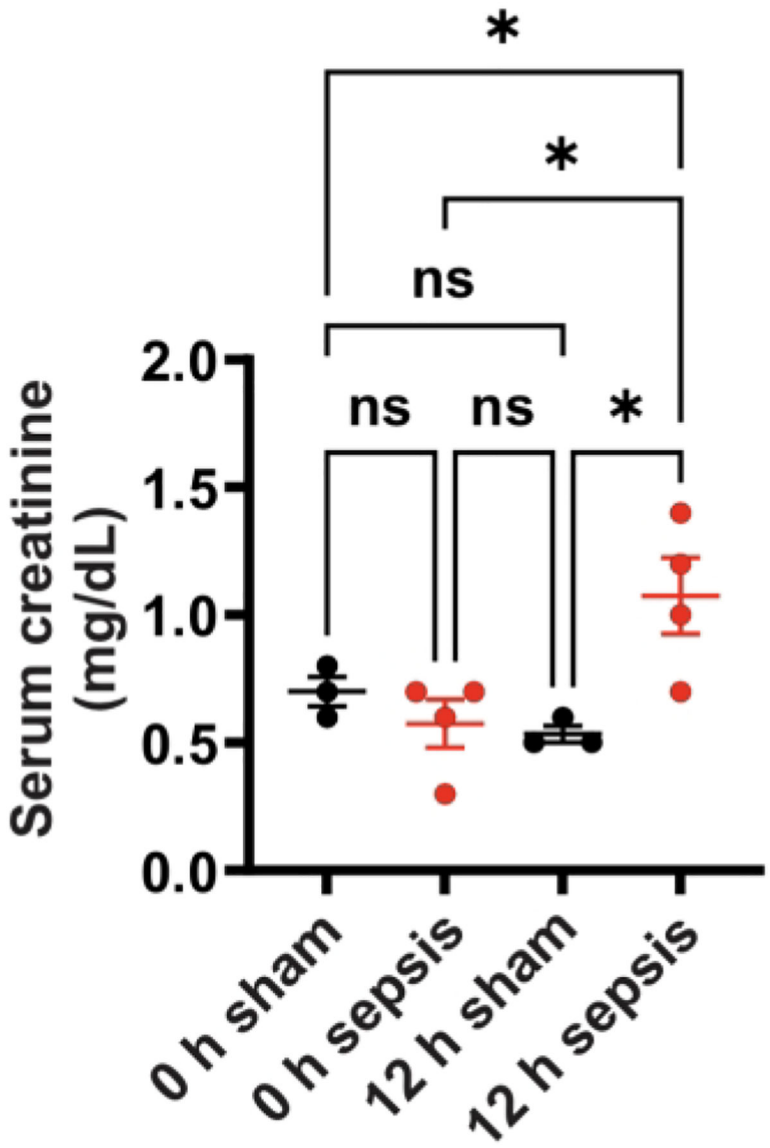


**Figure 6: Serum creatinine in mechanically ventilated sham and septic male piglets.**
(One-way ANOVA test).