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Modified Tail Vein and Penile Vein Puncture for Blood Sampling in the Rat Model

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Abstract

Blood samples are required in most experimental animal designs to assess various hematological parameters. This paper presents two procedures for blood collection in rats: the lateral tail vein puncture and the dorsal penile vein puncture, which offer significant advantages over other previously described techniques. This study shows that these two procedures allow for fast sampling (under 10 min) and yield sufficient blood volumes for most assays (202 µL ± 67.7 µL). The dorsal penile vein puncture must be done under anesthesia, whereas the lateral tail vein puncture can be done on a conscious, restrained animal.

Alternating these two techniques, therefore, enables blood draw in any situation. While it is always recommended for an operator to be assisted during a procedure to ensure animal welfare, these techniques require only a single operator, unlike most blood sampling methods that require two. Moreover, whereas these previously described methods (e.g., jugular stick, subclavian vein blood draw) require extensive prior training to avoid harm to or death of the animal, tail vein and dorsal penile vein puncture are rarely fatal. For all these reasons, and according to the context (e.g., for studies including male rats, during the perioperative or immediate postoperative period, for animals with thin tail veins), both techniques can be used alternately to enable repeated blood draws.

Introduction

Blood sampling is necessary for most animal studies, both *in vivo* and *in vitro*. In rats, as the frequency and amount of blood sampling can be significant, it is helpful to have different alternatives for collection. Various methods have been described in previous studies.

The most commonly used techniques are tail vein puncture and saphenous vein blood draw. Tail vein sampling is suitable for all rat strains. With proper training, the procedure is simple to perform and causes minimal distress to the animal[1]. Similarly, the saphenous vein blood draw, provided it is done properly, is also a quick and simple collection method. Neither method requires anesthesia, and both allow for repeated draws of small amounts of blood. However, the saphenous vein puncture usually yields a lower blood volume[1] and requires the presence of two people to leave one hindlimb exposed for puncture[2].

If large amounts of blood need to be collected from a single animal, cardiac puncture or puncture of the vena cava may be used (up to 10 mL of blood can be drawn from a 150 g rat with cardiac puncture[2]). These techniques require anesthesia and are terminal procedures. The animal must be euthanized after any of these two techniques[2]. The jugular stick is an alternative that can be used if large amounts of blood need to be collected in a study that has not yet reached its endpoint. However, this technique also requires significant technical skills to avoid harm to the animal; hence, its use should be limited[3].

Other techniques, such as the subclavian vein blood draw, do not need the use of anesthetics before blood collection and allow for repeated sampling of small volumes of blood. However, restrained handling and appropriate needle incision are required for this technique. An improper operation may result in animal pain or even mortality, and the training for this method may be fastidious[4].

Other anecdotal procedures include the orbital puncture and the sublingual vein puncture, both of which require an anesthetic, and neither are recommended nor widely used. Though previous studies have shown faster blood collection by orbital puncture than by tail vein puncture, it was found that orbital puncture under diethyl-ether anesthesia was less well tolerated than the latter method (based on the animals' excitation scores and urine production)[5]. Moreover, this method is highly influenced by the skill of the person who performs the procedure and is mainly performed by experienced veterinarians. Comparably, the sublingual vein puncture is less distressing and is recommended for repeated blood sampling[6]. However, this technique presents severe adverse effects such as reduced food and water intake, which can lead to the animal's death[7].

This study describes two methods used in our laboratory for repeated blood sampling. Tail vein puncture can be performed on a conscious animal, and the tissue damage and adverse effects are minimal. The modification of this technique in this study includes stabilizing the tail with the index and middle finger, which allows a single operator to perform the blood collection. The dorsal penile vein puncture has already been described for simple intravenous injections. This technique is performed under anesthesia and allows for a reliable blood source in case of difficulties with other methods (e.g., during the immediate postoperative period, with a small animal, when performing perioperative blood draw under anesthesia). Similar to tail vein sampling, the

injury at the puncture site will have a minor overall effect on the animal compared to the techniques mentioned above[8]. The aim of this methods paper is to offer inexperienced researchers simple and reliable blood sampling alternatives according to the context (e.g., for procedures done under anesthesia, for studies including male rats, for animals with thin tail veins).

Protocol

The procedures were performed on 3 month old male Lewis rats, each weighing 300-400 g. A total of 24 animals were included, with three puncture conditions: 12 rats underwent tail vein puncture without anesthesia (group TV without anesthesia), and another 12 rats were anesthetized to undergo both tail vein puncture (group TV with anesthesia) and penile vein puncture (group PV with anesthesia). All the procedures were approved and respected the Institutional Animal Care and Use Committee (IACUC) guidelines. All the animals were euthanized at the end of the study (after a 1 month follow-up) by carbon dioxide overdose. See the **Table of Materials** for details related to all the materials and instruments used in this protocol.

1. General guidelines

1. In line with the IACUC guidelines, ensure that the maximum blood volume drawn is no more than 10% of the total blood volume every 2 weeks[9]. For example, a rat of 300 g should have a total blood volume of approximately 19.2 mL. In the case of a protocol requiring four blood draws in the first week alone (day 0, day 1, day 3, day 7), limit the collection to a maximum of 250 µL of blood per sample.
2. For procedures performed under anesthesia, administer isoflurane *via* a precision vaporizer to anesthetize the animal. Induce anesthesia in a chamber with a dose of 3%-5% isoflurane for 5 min, and maintain using a dose of 1%-3% isoflurane through a nose cone during the procedure. Adjust the isoflurane level based on continuous monitoring of the respiratory rate. Verify whether sedation is sufficient by toe pinch before starting the procedure.
3. Do not leave the animal unattended during the procedure or until it has regained sufficient consciousness to maintain sternal recumbency.
4. After the blood collection, monitor the animal until full recovery before returning it to its cage, and do not introduce it to the company of other animals until fully recovered.
   NOTE: In agreement with veterinary services, no post-procedure pain medication was necessary after tail vein or penile vein puncture.

2. Blood draw from the penile vein

1. Preparation
   1. Prepare the following equipment: sterile gauze, gloves, alcohol wipes, a microcapillary blood collection EDTA tube (purple cap), and a 30 G insulin syringe (30 U or 50 U).
   2. Take the rat out of its cage, and put it in a chamber for induction with isoflurane *via* a precision vaporizer (dose: 3%-5%). Once the animal is sedated, transfer it to the procedure table, and lay the animal on its back with its nose placed in the nose cone to maintain the anesthesia. Monitor the respiratory rate, and adjust the isoflurane level accordingly

(maintenance dose: 1%-3%). Verify that the animal is sufficiently sedated by toe pinch before starting the procedure.

2. Blood sampling

    1. Move the plunger back and forth in the syringe several times to smoothen the withdrawal. Create negative pressure in the syringe by pulling on the plunger to remove a couple of microliters.

    2. With the help of the non-dominant hand, retract the foreskin from the end of the penis, and hold the glans between the index and thumb, pulling gently. The dorsal penile vein will appear as a superficial blue cord. See **Figure 1** and **Figure 2**.

    3. With the eye of the needle pointing upward, insert the insulin syringe into the vein at an angle of 35°. Once the needle has entered the vein, blood will flow into the syringe.

    4. Slowly withdraw the plunger of the syringe at a slow and steady rate to collect the desired volume.
    NOTE: Do not withdraw the plunger too fast, as this will cause the vein to collapse and stop the blood flow.

    5. If the blood flow decreases, rotate the needle slightly clockwise or counterclockwise.

    6. Remove the syringe. A blood drop will form on the puncture site, the aspiration of which will allow the collection of a few more microliters of blood in the case of a non-sterile procedure.

    7. If the first puncture fails, reinsert the needle more proximally on the vein.
    NOTE: Unlike for tail vein sampling, the iterative puncture of the dorsal penile vein is usually unsuccessful.

    8. Apply light pressure to the puncture site to stop the bleeding, and wipe the area with a new alcohol wipe.

    9. Place the penis back in its neutral position.

    10. Turn the isoflurane off, and monitor the rat until complete recovery. Return the rat to its cage.

3. Tail vein puncture

1. Preparation

    1. Prepare the following equipment: a plastic restraining holder, sterile gauze, gloves, alcohol wipes, a microcapillary blood collection EDTA tube (purple cap), and a 28 G 1/2 insulin syringe (30 U or 50 U).

    2. Take the rat out of its cage, and quickly secure it in a plastic restraining cone. Close the large end of the cone around the base of the tail. Ensure that the animal is comfortable and that breathing is unrestricted throughout the whole procedure.

    3. Dip the tail in warm water (37 °C) for about 1 min to dilate the vein. Dry the tail with a paper towel. Place the animal (in its restrainer) face down, with the tail lying on a heating pad.

    4. Select the right or left tail vein (blue line) for sampling by rotating the entire animal to either side (this avoids the twisting of the tail). Use the terminal third of the tail for blood vessel puncturing since the vessels become more superficial in this zone. The artery is ventral, and the two veins are lateral[10].

5. Wipe the tail with 70% ethanol wipes at the puncture site.
6. Place the tail on the edge of the heating pad to create an angle in the terminal third of the tail. This brings the vein to the surface and creates more space for taking the sample.

2. Blood sampling
   1. Move the plunger back and forth in the syringe several times to smoothen the withdrawal. Create negative pressure in the syringe by pulling on the plunger to remove a couple of microliters.
   2. With the help of the non-dominant index and middle finger, secure the tail flat on the heating pad. Place the middle finger proximally and the index finger distally, with the puncture site between these two fingers. Apply more pressure on the middle finger than on the index to secure the tail, occluding the vessel only proximally and allowing the blood to pool. See **Figure 1** and **Figure 3**.
   3. With the eye of the needle pointing upward, slide the insulin syringe against the index finger until it is inserted into the vein (this creates an angle of 35° between the needle and the tail). Once the needle has entered the vein, blood will flow into the syringe. At this point, release the pressure on the index and middle finger to ensure that the blood flow is not occluded.
   4. Slowly withdraw the plunger of the syringe at a steady rate to collect the desired volume.
      NOTE: Do not withdraw the plunger too fast; this will cause the vein to collapse and stop the blood flow.
   5. If the blood flow decreases, rotate the needle slightly in either direction.
   6. Remove the syringe from the tail. A blood drop will form on the tail's puncture site. The aspiration of this blood allows for the collection of a few more microliters of blood in the case of a non-sterile procedure.
   7. If the first puncture fails, reinsert the needle more proximally on the vein.
      NOTE: The vein becomes progressively more profound as it approaches the tail base. If there is no blood flow in the syringe, increase the angle between the syringe and the tail, or rotate the needle.
   8. Apply pressure to the puncture site to stop the bleeding, and wipe the area with a new alcohol wipe. Remove the rat from the plastic cone, and return it to its cage.

3. Tail vein puncture under anesthesia
   1. Perform step 2.1.1 and step 2.1.2 for inducing and maintaining anesthesia.
   2. Perform steps 3.1.3-3.2.7 for blood collection; see **Figure 1**.
   3. Perform step 2.2.10 for animal recovery.

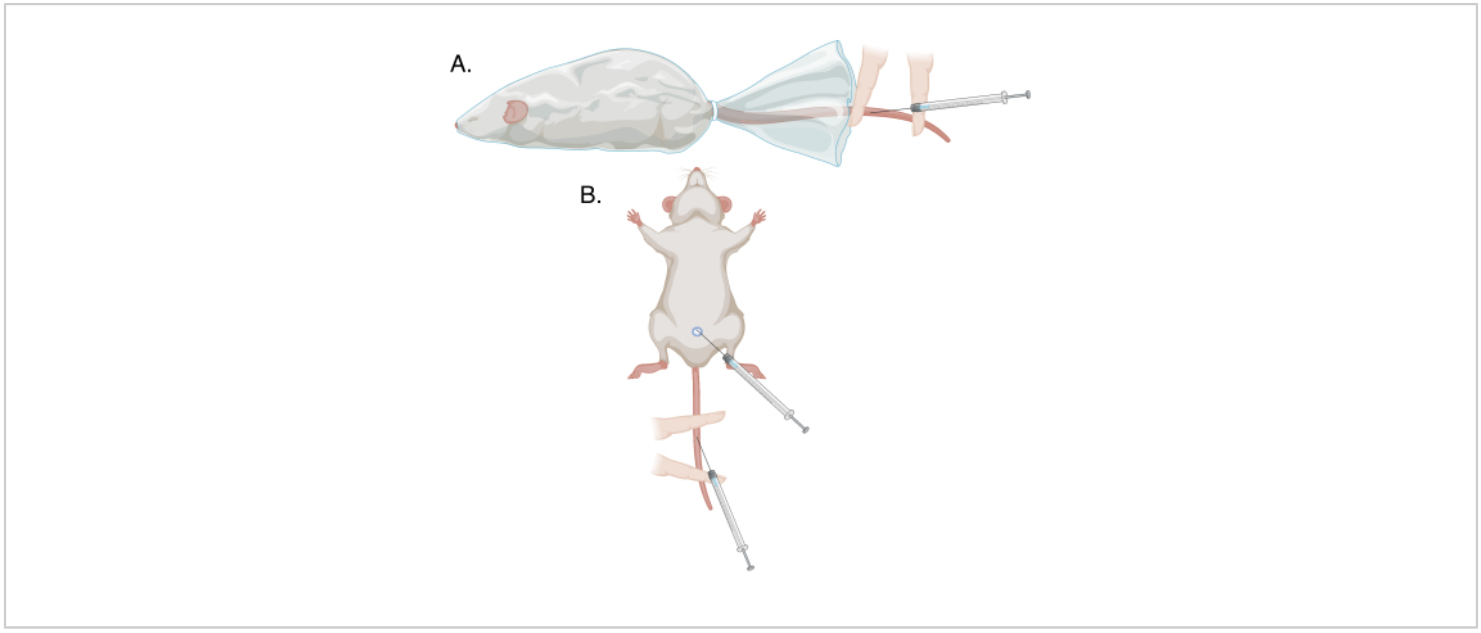

Figure 1: Schematics of the different puncture methods in this protocol. (**A**) Modified tail vein puncture on a conscious, restrained animal; (**B**) modified tail vein puncture and penile vein puncture under anesthesia. Please click here to view a larger version of this figure.

Representative Results

Success was defined as a blood draw yielding at least 100 µL of blood in under 10 min (from the puncture time to the end of the blood collection), and failure was defined as a blood draw yielding less than 100 µL of blood or taking more than 10 min to retrieve the required blood volume. A maximum of 250 µL of blood per sample was allowed. The statistical analyses were conducted using a one-way ANOVA test for multiple comparisons and the chi-squared test. The data were presented as mean value ± standard deviation, and $p < 0.05$ was used as the cut-off for determining statistical significance.

The comparison of the success rates showed similar results for tail vein puncture in conscious rats (92%) and penile vein puncture under anesthesia (83%)($p = 0.0543$), as shown in **Figure 4**. Interestingly, under anesthesia, the tail vein became very unreliable, and tail vein puncture under anesthesia had only a 25% success rate in this study, probably due to thinning of the vein. In the case of anesthesia, the penile vein puncture was more successful than the tail vein puncture for sampling ($p < 0.0001$).

We compared the blood volumes collected and the procedure durations among tail vein and dorsal penile vein puncture performed on rats under anesthesia and tail vein puncture performed on conscious rats. **Figure 5** shows that tail vein puncture without anesthesia (217.5 µL ± 69.04 µL) and penile vein under anesthesia (185.8 µL ± 66.4 µL) yielded comparable quantities of blood ($p = 0.4966$), and these volumes of blood were significantly higher than the volume collected with tail vein puncture under anesthesia (54.4 µL ± 68.8 µL) ($p < 0.0001$).

The duration of the procedure was similar in the penile vein puncture under anesthesia group (315.2 s ± 160 s) and the

tail vein puncture without anesthesia group (262.5 s ± 171 s) (p = 0.6632). **Figure 6** shows that sampling was performed in less than 6 min in both groups, whereas the tail vein puncture under anesthesia took more than 8 min (500.8 s ± 196 s) due to multiple failures (p < 0.0382).

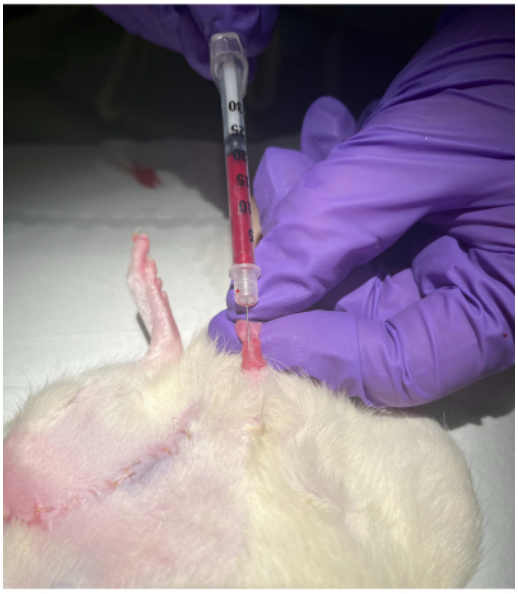

Figure 2: Dorsal penile vein puncture method. Please click here to view a larger version of this figure.

Figure 3: Modified tail vein puncture method. Note that the tail is held down and the puncture site is located between the index and middle fingers. The syringe should rest and slide against the index finger to maintain a stable puncture angle. The use of the non-dominant hand allows for the stabilization of the tail on a conscious animal. Please click here to view a larger version of this figure.

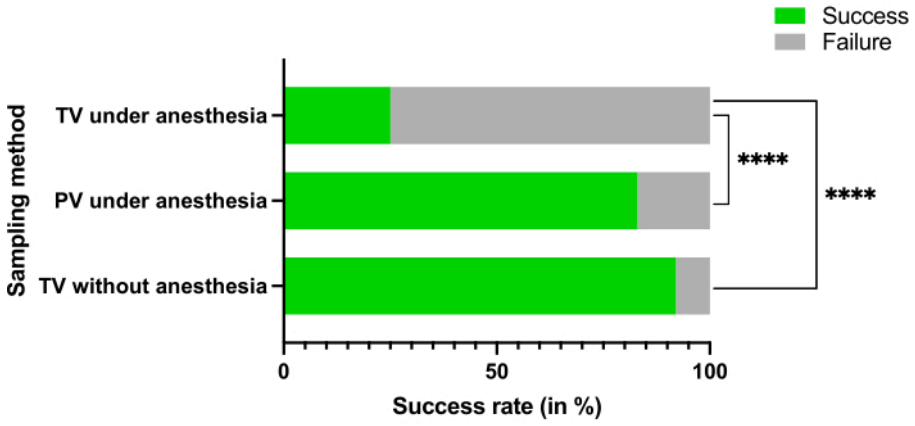

Figure 4: Success rates with tail vein puncture under anesthesia, penile vein puncture under anesthesia, and tail vein puncture without anesthesia. **** $p < 0.0001$ with the chi-squared test. Abbreviations: TV = tail vein; PV = penile vein. Please click here to view a larger version of this figure.

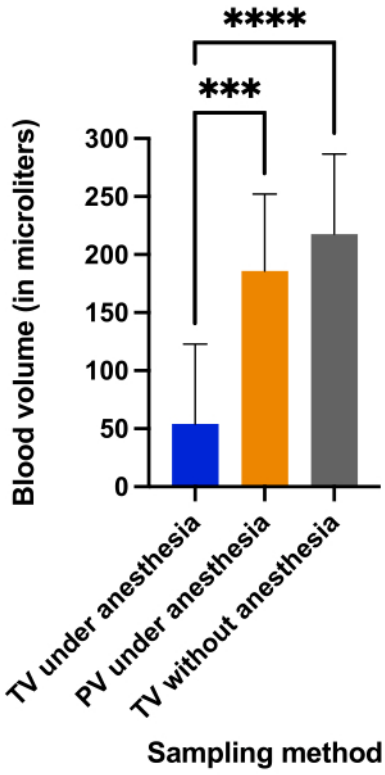

Figure 5: Comparison of the blood volumes (in µL) obtained in the three groups. ***p < 0.001; ****p < 0.0001 with ANOVA analysis for multiple comparisons. Please click here to view a larger version of this figure.

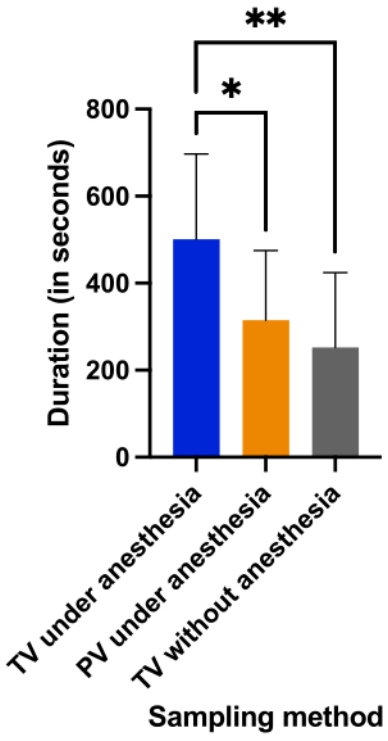

Figure 6: Comparison of the sampling duration (in seconds), defined as the time from the moment of puncture to the end of the blood draw, in the three groups. Failure was defined as a blood draw duration lasting longer than 600 s (10 min). *p < 0.05; **p < 0.01 with ANOVA analysis for multiple comparisons. Please click here to view a larger version of this figure.

Discussion

The tail vein puncture is an efficient method to obtain blood from a conscious rat. However, when an animal is under anesthesia, the effect of isoflurane can lead to vessel spasms and make tail vein puncture unsuitable[11]. As shown in this study, an alternative in this situation is to collect blood from the penile vein, which is more successful and yields a significantly greater volume of blood in less time. It is important to remember that in the case of failure with this method on the first try, subsequent attempts may be unsuccessful. In contrast, tail vein puncture allows for several subsequent punctures in case of difficulty on the first try (there are two veins, and puncturing higher up on the tail can be attempted)[1]. However, iterative punctures can cause hemolysis in the sample, which distorts the results due to the release of hemoglobin and the internal components of erythrocyte membranes. This is especially true if the plasma is analyzed[12]. Avoiding multiple punctures and obtaining the sample in a single blood draw is preferable.

In this work, the blood sampling did not exceed 250 µL per sample in accordance with this protocol and the IACUC guidelines to respect animal welfare in the case of multiple sampling. Advances in bioanalytical techniques have enabled the use of microsamples of less than 50 µL to assess the

blood biochemistry and metabolic parameters [13]. Therefore, 250 µL is sufficient to conclude that both methods are efficient for future studies. However, both the tail vein and the penile vein are small vessels and do not allow for the collection of large amounts of blood. These described methods are appropriate for repeated sampling and the monitoring of living animals. If large quantities of blood are necessary (for example, for end-of-study procedures), other methods yielding more blood-such as cardiac puncture-should be considered.

These described procedures are two among many; our choice of procedure was motivated by a few advantages. Both these techniques can be done by a single operator. Using the index and middle finger to stabilize the tail vein in the modified tail vein technique makes complementary human restraining unnecessary. However, it is necessary to assess the animal's welfare, and assistance by a professional (e.g., a veterinarian or veterinary technician) is always recommended to avoid subjecting the animal to unnecessary pain or distress[2].

Moreover, previously described methods (e.g., jugular stick, subclavian vein blood draw) require extensive prior training to avoid harm to or death of the animal. In contrast, tail vein or dorsal penile vein puncture are rarely fatal to the rat, even if not well executed. This study had some failures with both methods, but no other adverse effects or deaths were observed. Some cases of urine retention after dorsal penile injections have been reported, but it is unclear whether the injection itself or the agent injected is responsible for this outcome. Neither this adverse effect nor abnormal healing or infection at the puncture site were noted during the study period. Furthermore, the daily assessments of the conditions of the animals did not reveal pain or distress in any groups (no porphyrin staining, no weight loss, animals judged to be comfortable by the research team and veterinary professionals). However, prolonged restraining and multiple punctures can cause animal distress. To avoid this, the needle should be rotated when blood flow slows down instead of repeating the puncture. Properly warming the tail with hot water and a heating pad to vasodilate the tail vein, as well as practicing these methods, are recommended to reduce the restraining time.

The tail vein puncture and dorsal penile vein puncture methods allow for fast sampling (under 6 min) and yield sufficient blood volumes for most assays. When done on a conscious animal, tail vein puncture is an efficient and reliable method to obtain blood. However, on a sedated animal, anesthetic agents tend to cause vessel spasms, and the tail vein is subject to important thinning[11]. In this scenario, penile dorsal vein puncture provides a better success rate than tail vein puncture, which tends to be unreliable for blood collection. However, one limitation of the penile vein blood draw is that it can only be performed on male rats and is, therefore, unsuitable for female rat studies. Therefore, according to the context (i.e., the sex of the animal, perioperative or post-operative blood draw, animals with thin tail veins), both penile vein puncture and tail vein puncture can be used alternately for repeated blood draws, even by researchers with little to no experience with animal studies.

Disclosures

None of the authors have any conflicts of interest to declare.

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