Methods for Assessment of the Glomerular Filtration Rate in Laboratory Animals

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Abstract

Background: The glomerular filtration rate (GFR), as the benchmark of renal function, has been widely used in clinical practice and basic medical research. Currently, most researchers still rely on endogenous markers, such as plasma creatinine, blood urea nitrogen, and cystatin C, to evaluate renal function in laboratory animals. While inexpensive and simple to use, methods based on endogenous markers are often inaccurate and susceptible to several internal physiological factors. Thus, it is necessary to establish a method to precisely assess the GFR, especially when detecting early changes in GFR during acute kidney injury, and hyperfiltration usually caused by pregnancy or diabetic nephropathy. In addition, laboratory animals have higher tolerance for invasive procedures than humans, allowing novel technologies to be applied on them for GFR monitoring. In recent years, significant progress has been made in developing new methods to assess GFR in animals. However, no publication has reviewed these techniques. Summary: This article summarized the majority of methods used to assess the GFR in animals in recent decades and discussed their working principles, workflows, advantages, and limitations, providing a wealth of reference and information for researchers interested in studying the kidney function in animals and developing techniques to monitor the GFR.

Introduction

The glomerular filtration rate (GFR), as the benchmark of renal function [1], has been widely used in both clinical practice and basic medical research. In clinical practice, the estimated GFR (eGFR), which is derived from plasma creatinine, is commonly used to define and stage acute kidney injury (AKI) [2] and chronic kidney disease (CKD) [3]. However, clinical data based on large samples indicate that the eGFR is less accurate when the GFR is close to the normal range, making early detection of kidney injury difficult [4]. This can be attributed to the compensatory hyperfiltration of the remaining functioning nephrons, secretion of creatinine in renal tubules, and extrarenal elimination of creatinine as the GFR declines [5]. In basic research, this inaccuracy persists. Plasma creatinine and urea nitrogen are unable to not only detect hyperfiltration that occurs during diabetes and pregnancy but also determine the renal reserve, which is a clinical...
indicator for early diagnosis [6–10]. Additional factors, such as blood draw volume, anesthesia, and stress, should also be considered during animal GFR measurements. GFR monitoring in laboratory animals should reflect real-time GFR [11, 12], detect mild changes in the GFR [12], and not be stressful [13]. Using laboratory animals is beneficial as invasive interventions, such as bladder catheter insertion, can be performed [11]. Although significant progress has been made regarding the development of methods to assess GFR in laboratory animals, a standard does not exist.

This review article aims to achieve the following: (1) summarize and evaluate existing methods for GFR assessment in laboratory animals, including their principles, workflow, strengths, and limitations; (2) describe the progress of GFR assessment methods on nonmammalian animals; and (3) propose the future direction of the development of GFR assessment technology as well as potential influencing factors. We hope that this review can provide extensive and valuable information for researchers interested in studying the kidney function of animals and developing GFR monitoring technology.

**Clearance Rate and the Gold Standard of Measurement**

Clearance refers to the amount of plasma that is completely cleared from a substance over a set amount of time [1, 14]. The clearance of an ideal filtration marker equals the GFR. As described in the book *Brenner and Rector’s The Kidney*, such a marker should be “distributed freely and instantaneously throughout the extracellular space, not bound to plasma proteins, freely filtered at the glomerulus, not secreted or reabsorbed at the tubules, eliminated wholly by the kidney, resistant to degradation, and easy and inexpensive to measure” [5].

However, the ideal glomerular filtration marker does not exist. Inulin, which is the classical filtration marker, is not used in clinical practice as it does not readily dissolve in aqueous solutions, is difficult to measure, and has a limited supply [15].

The clearance rate can be calculated based on one bolus intravenous infusion or constant continuous infusion of an exogenous filtration marker. The route of clearance may be divided into plasma clearance and urinary clearance. The GFR is calculated from the plasma clearance using the following formula [1, 15]:

\[
Cl_x = \frac{A_x}{P_x},
\]

where \(Cl_x\) means the clearance of \(x\) substance, \(A_x\) is the amount of the marker administered, and \(P_x\) is the curve of plasma concentration over time.

Exogenous filtration markers do not enter cells; they diffuse from the plasma into the extracellular space (fast component) and are subsequently excreted by kidneys (slow component). Plasma clearance is best estimated using a two-compartment model to fit the clearance curve with multiple blood samplings.

Urinary clearance first requires a constant and continuous intravenous infusion of exogenous markers with a low flow rate. After a set period (usually dozens of minutes), the plasma concentration of the marker will plateau. Blood and urine samples are then collected to determine the concentration, and the GFR is then calculated using the following formula [1, 15]:

\[
Cl_x = \frac{Ur_x \times V}{P_x},
\]

where \(Cl_x\) is the clearance of substance \(x\), \(Ur_x\) is the urine concentration of \(X\), \(V\) is the urine flow rate, and \(P_x\) is the plasma concentration of \(X\).

Inulin urinary clearance, which is the gold standard, was used in a few publications, which were mainly methodological studies [16–18]. However, this method has not been utilized in routine clinical practice [5, 15].

**Utilizing Plasma Levels of Endogenous Markers to Evaluate GFR**

Endogenous markers, such as creatinine, urea nitrogen, and cystatin C, are often used to assess renal function in laboratory animals. However, unlike the human eGFR algorithm, the correction algorithm to convert plasma concentration to GFR for laboratory animals is rare [19]. Most scientists rely on the changes in the plasma concentration of the marker to evaluate the GFR. However, studies have shown that the plasma concentration of endogenous markers is not always positively correlated with the measured GFR, especially when kidney injury is not severe [5, 20]. Furthermore, they are not direct indicators of the remaining renal filtration function, making it difficult to determine whether kidney failure has occurred based on the increased concentration. Moreover, this method does not reflect hyperfiltration [21, 22].

Creatinine, as a product of normal muscle metabolism, is nonenzymatically converted from creatine and phosphocreatine at an almost steady rate (approximately 2% of total creatine per day). Since it is not bound to plas-
ma proteins, it is freely filtered by the kidneys; hence, creatinine has become a widely used renal marker by physicians and researchers [5]. However, it is secreted by the renal tubules. According to reports, 10%–40% of creatinine can be secreted by the renal tubules and may result in an overestimated true GFR [23]. Another study has shown that five transporters are likely involved in the renal clearance of creatinine: OAT2, OCT2, OCT3, MATE1, and MATE2-K [24]. Drugs that block creatinine secretion, such as cimetidine, trimethoprim, ranolazine, pyrimethamine, dronedarone, and tenofovir, can cause slight elevation in the level of serum creatinine, which reflects a loss of tubular creatinine secretion instead of a change in GFR [14]. Additionally, the creatinine level is influenced by factors such as muscle mass, diet, and exercise. It has been proven that creatinine is poorly correlated with GFR values obtained by golden methods [20, 25].

Cystatin C, a low-molecular-weight (13 kD) protein, is produced by nucleated cells at a constant rate [15]. Cystatin C is freely filtered in the glomerulus, reabsorbed and catabolized, and rarely excreted in the urine [26]. Urea nitrogen is also extensively used to calculate the GFR; however, it is also influenced by many factors. Insufficient volume (prerenal azotemia), gastrointestinal bleeding, corticosteroids or cytotoxic agents, high-protein diets, obstructive urinary tract disease, and sepsis reportedly increase the level of blood urea nitrogen disproportionately. In contrast, low-protein diets and liver disease can cause plasma levels of blood urea nitrogen to disproportionately decrease [27]. Similar to creatinine, urea nitrogen is also not sensitive enough to accurately measure the GFR [28, 29].

Utilizing the Clearance Rate of Exogenous Markers to Calculate the GFR

In this part, we will summarize several exogenous markers, including inulin, fluorescein isothiocyanate (FITC)-sinistrin, zwitterion 800-1, iohexol, radiolabeled markers, and unlabeled radiocounter agents. All these markers can be detected by different methods to calculate their clearance rates, which equates to the GFR.

Inulin

Previously, inulin (a plant starch) of MW 5,200, is a straight-chain polysaccharide, and its structure depends on its source. For example, chicory inulin is composed of a mixture of oligomers and polymers in which the degree of polymerization varies from 2 to 60 units, with an average of 12 [32]. Inulin is now no longer available; instead, polyfructans are used. However, the term “inulin” is still used. Inulin is only freely filtered by the glomerulus, bound to plasma protein, and not absorbed or secreted by the renal tubules, which makes it an ideal marker [33]. In animal experiments, the following inulin-based methods have been developed: (1) a fluorescence spectrophotometer was developed through the coupling of FITC fluorescein to inulin to detect the plasma concentration of inulin [34]; however, due to poor solubility, inulin needs to be dissolved at high temperatures and dialyzed for 24 h to remove residual FITC [34]. (2) To simplify the detection of inulin by HPLC, a fluorescence density detector was placed on a pig’s tail to record the reflected light intensity of a newly developed Cy7.5-inulin at 805 nm, thereby obtaining a continuous clearance curve [18]. The GFR is then calculated by dividing the Cy7.5-inulin dose by the area under the curve. However, this study only obtained one GFR value of 120 mL/min. Although it is close to other published GFRs of pigs with similar body weight (130 mL/min, 70 kg pigs), no other methodological study has been implemented. (3) To measure inulin clearance in unrestrained rats, a study implanted an osmotic pump instead of a syringe pump to infuse inulin continuously [35].
**FITC-Sinistrin**

Sinistrin, which is a readily soluble preparation of polyfructosan with side branching, has better solubility than inulin without weighing significantly different from inulin. The clearance of sinistrin is identical to that of inulin [36–38]. FITC-sinistrin was first reported as a GFR marker in 2005 [38]. Currently, MediBeacon is selling its commercial reagents with a miniature fluorescence density detector to form a set of transcutaneous GFR (tGFR) monitoring systems for the determination of GFR in laboratory animals. After one bolus of intravenous injection of FITC-sinistrin, the optical device can rapidly measure the pharmacokinetics (PK) of the marker through the skin [39]. This method mainly detects green light reflected by FITC in the extracellular space because hemoglobin in blood vessels absorbs most of the reflected green light at 520 nm [40]. Once reaching an equilibrium state between the blood vessels and interstitial space, the excretion rate of sinistrin inside and outside the blood vessels will remain the same [40, 41]. Therefore, the classic two-compartment PK does not fit the whole curve, especially at the distribution stage. In 2016, MediBeacon developed a new three-compartment model with baseline correction that complemented the complete kinetic curve and corrected the baseline shifts observed during measurement. Possible reasons for shifts in the background signal include photo bleaching of the skin, autofluorescence, changes in the physiological state of the animals during the measurements, or effects arising from the device attachment. After applying the new 3-compartment kinetic model with a modulated baseline (tGFR3cp,b,m), tGFR measurements in rats reached comparable precision as those of the gold standard technique based on a constant infusion clearance.

The method has already been validated in different species and successfully applied in various models of renal pathology [40, 42–45]. Moreover, it is suitable for sequential measurements on the same individual to determine the progress of renal function as it is minimally invasive. Furthermore, it is sensitive enough to detect early changes in AKI and hyperfiltration during diabetes or gestation [22, 31]. It also avoids the effects of long-term anesthesia during GFR measurement. Interestingly, transcutaneous fluorescence technology can determine when a steady-state condition has been reached in a constant infusion method, which helps determine the infusion rate, and equilibration time [46].

However, the method seems to be imperfect. One study indicated that the tGFR results were significantly different from those of the plasma method in obese mice rather than lean mice [42]. Additionally, there was some variability when 3–4 simultaneously measured tGFRs in 11 Sprague Dawley rats were compared [41]. Besides, it can only calculate the $t_{1/2}$ rather than the GFR when used on nonrodent animals [43, 47]. Unfortunately, methodology validations on these species seem rare.

**ZW800-1**

ZW800-1 is a near-infrared fluorophore synthesized mainly by using a quaternary ammonium salt on the indocyanine skeleton, with a net charge of 0. This molecule was designed to replace the clinical contrast agent indocyanine green, which is extensively used in image-guided surgery to evaluate tumor tissue resection margins and photothermal therapy [48–51]. Due to its unique physicochemical and optical properties, ZW800-1 clearance can calculate the GFR as it is only excreted by the kidney and is unbound to plasma proteins. As ZW800-1 is a near-infrared fluorophore, its emission cannot be absorbed by tissues or blood components, exhibiting a low background [52]. ZW800-1 clearance also showed good agreement with the FITC-inulin clearance on the same individuals. However, methodological validation data, such as a comparison of transdermal and plasma methods, are scarce. In addition, ZW800-1 continuously decays in plasma. Although a correction algorithm was developed, its consistency with FITC-inulin did not significantly improve. Additionally, this method requires continuous anesthesia in animals to obtain multiple fluorescence images. Interestingly, the rate of distribution of ZW800-1 in the body may also reflect cardiac function, making the ascending portion of the clearance curve more meaningful [53].

**Iohexol**

Iohexol (Omnipaque), is a low-molecular-weight (821 Da) and nonionic intravenous contrast agent with a relatively low osmotic pressure that is often used in radiology research in the USA. Despite its low toxicity, only 1/50 to 1/10 of the radiologic dose is required for GFR determination [5]. Since iohexol is almost completely eliminated by glomerular filtration and is not reabsorbed, secreted, or metabolized by the kidneys, it may be considered as an ideal filtration marker. Consequently, it has been used as a substitute for inulin and radioactive isotopes. Even in advanced chronic renal failure, it has minimal protein binding and negligible extrarenal clearance [54–56].

Whether in clinical or basic research, iohexol clearance shows excellent consistency with inulin clearance [55]. However, GFR determination using iohexol re-
Glomerular Filtration Rate Assessments

Radiolabeled Markers

Previously, radiolabeled markers have been used to noninvasively evaluate renal function, especially when determining a single kidney’s GFR. $^{99m}$Tc-DTPA, $^{51}$Cr-EDTA, and $^{125}$I-labeled iodotitanate have been extensively studied, and their clearance has been compared with inulin renal clearance. These low-molecular-weight compounds are freely filtered by the glomerulus but differ slightly in protein binding or renal disposition [54].

$^{99m}$Tc-DTPA (393 Da) is currently the most used radiolabeled marker. It is only filtered by the glomerulus, and it is not absorbed or secreted by the renal tubules. However, it has a binding rate of 5%–10% to plasma proteins, and $^{99m}$Tc may unpredictably dissociate from DTPA, resulting in an underestimation of the GFR. For a 20-mCi (740-MBq) dose of $^{99m}$Tc-DTPA, the radiation exposure of the kidneys and bladder is 1.8 and 2.3 rad, respectively [59]. Therefore, its radiotoxicity on the kidneys should be considered, especially when long-term longitudinal measurements are required.

$^{51}$Cr-EDTA (292 Da) appears to have minimal protein binding, and its plasma clearance correlates well with that of inulin. However, EDTA can be reabsorbed by the renal tubules, leading to an underestimation of the GFR [5, 15, 60]. $^{125}$I-Iothalamate (614 Da) is a high-osmolar anionic contrast agent with some protein binding that undergoes active secretion by the proximal tubules, leading to an overestimation of plasma clearance [54].

The conventional workflow of a radionuclide test on humans is performing a one bolus injection of a nuclide followed by image scanning every 1–3 s within the first minute through a gamma camera such as PET or SPET. The interval data are used to evaluate renal perfusion. In the next 30 min, one frame of image is scanned every minute to evaluate the clearance rate, but the accuracy is easily affected by the kidney depth and background correction, and the error will increase in patients with poor renal function [61, 62]. For use in animals, the workflow is basically similar to that in humans except that the scanning parameter needs to be adjusted according to the animal size.

Some studies have shown that the ratio of $^{99m}$Tc uptake by the kidneys is positively correlated with and estimates the GFR. However, this seems inaccurate. In one study, there was a significant difference when different time intervals and background regions of interest were chosen as determining factors when calculating the glomerular filtration ratio based on the uptake percentage of $^{99m}$Tc-DTPA [63]. It was also proven that following intravenous saline administration, changes in the time to peak activity and mean renal transit time may create inaccuracies in GFR estimates based on the conventional regression formula, thereby underestimating the true GFR value [64]. Intravenous fluid therapy had reportedly similar influence in three infusion modes in cats [65]. However, the mechanism remains unclear, and this issue seems to be more important in clinical practice, especially when evaluating the renal function in patients undergoing active diuretic or intravenous infusion therapy.

Unlabeled Radiocontrast Agents

Common unlabeled radiocontrast agents for evaluating GFR include iohexol iodine and gadolinium contrast agents. As mentioned above, iohexol is an almost ideal GFR marker, and its PK is equivalent to that of inulin. Hence, iohexol can be used as an iodinated contrast agent in radiography and computer tomography (CT). Meanwhile, gadolinium contrast agents, such as gadolinium meglumine and gadolinium dimethylamine, are generally applied in conjunction with MRI to evaluate the GFR.

The conventional renal CT protocol includes three steps: baseline precontrast imaging, single-slice dynamic imaging, and postcontrast imaging [66]. Similar to a radionuclide scan, a CT scan with iohexol can obtain GFRs and morphological data of one or two kidneys. An enhanced CT scan has a higher resolution than a radionuclide scan, but motion artifacts caused by breathing are an issue. Hence, animals require continuous anesthesia during a CT scan. In a recent study, iopamidol-enhanced CT-GFR and FITC-sinistrin transdermal measurements were performed simultaneously on the same mice and compared. Although both methods reflected a significant difference in the GFR between the normal and kidney injury groups, the measured GFR of the normal group was significantly lower than that of the latter [66]. A similar result was found in another study that hypothesized that altered renal blood flow, hematocrit of the small vessels, and nephrotoxicity play a role in the underestimation of GFR as measured by contrast-enhanced CT [67].

Unlike CT, MRI is not radiotoxic and has excellent soft tissue contrast, high spatial resolution, safety, and short
image acquisition time. It also has several functional indicators, such as the single/global kidney GFR, renal blood volume, plasma average transit time, tubular flow, and tubular average transit time [68]. A study has indicated that in some cases, the MRI-GFR highly agrees with the optical-GFR (FITC-sinistrin percutaneous fluorescence method) [69]. Meanwhile, another study showed that the MRI-GFR results were almost twice as high as those of optical-GFR due to unknown factors [70]. Regarding the specifications of MRI-GFR measurement, some researchers have proposed the demand for standardized procedures, such as the anesthesia protocol, automatic contrast injection, regions of interest location and size, and time interval for RPP integration. Moreover, a cyst/fibrosis segmentation algorithm should be developed to not only separate a cyst from healthy kidney tissue but to also calculate the three-dimensional volume of the kidney to improve the accuracy and reduce the influence of several factors [70, 71].

**Other Methods**

**In vivo Heart Fluorescence Imaging**

This method was first reported by the PerkinElmer company. After one bolus intravenous injection of inulin labeled with a near-infrared fluorophore (GFR-Vivo 680), noninvasive fluorescence molecular tomography heart imaging quantified the blood PK over a period of time and calculated the GFR using two-compartment PK modeling. In this study, the imaging results correlated well with ex vivo plasma microplate assays for inulin blood kinetics. However, the sample size was small (n = 3), and the PK curve of the fluorescent signal of the kidneys was inconsistent with that of the plasma concentration. The author did not explain possible reasons for this difference [72]. Additionally, this method requires continuous anesthesia, which may influence the GFR. Furthermore, as this method utilizes the fluorescence imaging PK value of the heart to calculate the GFR, its accuracy should be doubted when animals have heart disease or functional abnormalities such as myocardial infarction. There are also several factors that need to be validated, such as displacement caused by twitches during anesthesia and skin color. In a study published in 2021, this method was further improved: 15 μL of blood was drawn at different time points, placing them on a microplate for fluorescence imaging, and fluorescence intensity in the plasma was then detected and converted into the concentration of the fluorescent marker by using an in vitro standard curve. This in vitro method avoids the effects of continuous anesthesia and skin color [73].

**In vivo Multiphoton High-Resolution Fluorescence Imaging Technology**

In vivo multiphoton high-resolution fluorescence imaging technology utilizes a multiphoton microscope to perform fluorescence imaging of deep tissues. This method has several advantages as it is noninvasive, has low phototoxicity, and allows for dynamic visualization. In 2006, this technique was used to measure the GFR of a single nephron (snGFR) [74]. Based on the classic micro-puncture technology [75], multiphoton fluorescence imaging first selects an optical section with a proximal tubule at least 100-μm long, injects an extracellular fluid marker (fluorescent yellow) through the femoral vein, and starts recording at the proximal tubule. It only takes 10 s to calculate the transmission time of the two fluorescence peaks. The fluid volume in the small tube is then calculated [((length × [diameter/2]² × π)/3)], and the snGFR is obtained. The snGFR obtained from this method is not significantly different from that obtained by the FITC-inulin constant bolus clearance method. By adding multiple fluorescent substances (dextran-rhodamine B conjugate, lucifer yellow, FITC-inulin), multiple functional indicators could be detected aside from the GFR, including glomerular permeability, renal tubular fluid and blood flow, urine concentration/dilution, and renin content and release. Moreover, this method can directly observe fluid changes in one nephron during the GFR measurement [74].

Another method, called ratio microscopy imaging technology, utilizes a two-photon microscope to monitor fluorescence changes to measure the GFR. After one bolus intravenous injection of two fluorescent substances (3–5-kDa FITC-inulin and 500-kDa Texas red-dextran), the signals of the macromolecular fluorescent substances in blood vessels are maintained for 75 min. The ratio of the two fluorescent signals, which reflects the change in plasma FITC-inulin concentration, is then detected to calculate the FITC-inulin plasma clearance rate. The whole process can be completed within 5 min [76]. Although this ratiometric technique eliminates the need for continuous infusion and urine collection, no other report has applied this method to evaluate renal function.

Currently, only few studies have utilized this method, probably because of its high cost. A recent study has improved this method by applying three-dimensional...
dataset modeling to estimate the actual PT volume instead of two-dimensional volume estimation [77]. One study has suggested that multiphoton imaging has high sensitivity in the early stages of AKI [76]. Despite its unique advantages, further validation studies, such as a comparison with inulin clearance, should be performed, especially when GFR exceeds the normal thresholds.

**Effects of Anesthesia on Animal GFR**

Since many methods require animals to be anesthetized during GFR measurements, it is necessary to understand the possible effects of anesthesia on the GFR. However, only few peer-reviewed articles have focused on this issue. Herein, we have summarized data from these studies to provide a reference for researchers when choosing appropriate anesthetics (Table 1) [78–88]. As shown in the table, most of the anesthetics can reduce the GFR. Regarding isoflurane, it is considered a safe anesthetic that results in few complications; however, data regarding this are not available. The further development of GFR assessment methods may result in this issue gaining more attention.

**Nonmammalian GFR Measurement**

With the development of laboratory animal science and comparative medicine, nonmammals have also been used in multiple research areas. Currently, only a few studies have assessed the GFR on nonmammals, such as fish [89], reptiles [90, 91], and birds [92, 93], and ferrets [94–96]. In these studies, the clearance of exogenous markers (such as $^{51}$Cr-EDTA, inulin, $^{99m}$Tc-DTPA clearance, $^{14}$C sodium ferrocyanide, and iohexol) using a single bolus or constant intravenous infusion was often utilized to evaluate renal function. To collect blood and urine samples from fish, researchers resorted to intubation [89]. For birds, direct plantar vein puncture or brachial artery puncture was the common method [92, 93]. As for the mathematical algorithm, one-compartment [94], two-compartment, or noncompartment models [92, 94, 95] have been used to fit clearance curves. However, few studies have compared these methods to the gold standard.

Morphologically, mammalian kidneys are very different from those of reptiles or birds. For example, two types of nephrons can be found in bird kidneys: the cortical or reptile type (70%–90%, simple nephrons without the loop of Henle) and the medullary or mammalian type (10%–30%, a complex nephron with the loop of Henle). Therefore, it may be not appropriate to use mammalian GFR assessment methods to assess nonmammalian GFR. In addition, creatinine in birds can be secreted by the renal tubules (when plasma creatinine levels increase) and re-absorbed (when plasma levels are normal) [95]. For birds, there is evidence that GFR reduction can be caused by either kidney disease or a normal physiological response to dehydration [92]. Therefore, more studies are needed to explore nonmammalian GFR assessment methods. Additionally, these studies should also consider the unique morphology, physiological function, and inconvenience in collecting blood or urine samples in these animals.

| Anesthesia drug                      | Animal species | Impact on GFR          |
|--------------------------------------|----------------|------------------------|
| Propofol                             | Rat            | Decrease by 20%–30%    |
| Fentanyl-fluanisone                   | Rat            | Decrease by 20%–30%    |
| Thiopental sodium                    | Rat            | Decrease by 20%–30%    |
| Medetomidine-butorphanol              | Dog            | Increase               |
| Medetomidine-butorphanol-atropine    | Dog            | None                   |
| Etomidate                            | Dog            | None                   |
| Propofol                             | Dog            | None                   |
| Thiopental sodium                    | Dog            | None                   |
| Carprofen-medetomidine-propofol-isoflurane | Dog        | None                   |
| Ketoprofen                           | Piglet         | Decrease               |
| Propofol                             | Cat            | Decrease by <30%       |
| Furosemide                           | Rat            | Decrease by <30%       |

Table 1. Effects of anesthetics on animal GFR
| Method                                                                 | Marker                                                                 | Instrument                                  | Pros                                      | Cons                                                     | Publication |
|------------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------|-------------------------------------------|----------------------------------------------------------|-------------|
| Utilizing plasma levels of endogenous markers to evaluate GFR          | Endogenous markers (creatinine, urea nitrogen, cystatin C)              | Biochemical analyzer or HPLC or mass spectrometry | Convenient                               | Inaccurate                                              | ****        |
| Utilizing the clearance rate of exogenous markers to calculate GFR     | Inulin                                                                 | HPLC                                        | Highly accurate                           | Invasive (require multiple blood samplings or urine collections); not easy to dissolve | ****        |
|                                                                        | FITC-sinistrin                                                        | Fluorescence density detector               | Accurate; noninvasive; unstrained         | Need more methodological validation (obese animals, repeatability) | ***         |
|                                                                        | ZW800-1                                                               | Fluorescence imaging system                 | Noninvasive; potential to monitor cardiac function | Need more methodological validation                    | *           |
|                                                                        | Iohexol                                                               | HPLC or mass spectrometry                   | Accurate                                 | Invasive require multiple blood samplings or urine collections | ***         |
|                                                                        | Radiolabeled markers (59mTc-labeled DTPA, 51Cr-EDTA, and 125I-labeled iodotitanate) | Gamma camera                                | Noninvasive; capable to detect single-kidney GFR | Nephrotoxicity; influence by factors (e.g., ROI, background, artifacts) | ***         |
|                                                                        | Unlabeled radiocontrast agents (iohexol iodine contrast agent and gadolinium contrast agent) | CT or MRI                                   | Noninvasive; capable to detect single-kidney GFR; simultaneously obtain morphological information | Influenced by factors (e.g., ROI, background, artifacts) | ***         |
| Other methods                                                          | In vivo heart fluorescence imaging (GFR-Vivo 680)                      | Fluorescence imaging system                 | Noninvasive                               | Need more methodological validation                      | *           |
|                                                                        | In vivo multiphoton high-resolution fluorescence imaging (dextran-rhodamine B conjugate, lucifer yellow, FITC-inulin) | In vivo multiphoton high-resolution fluorescence microscope | Capable to detect single-nephron GFR; simultaneously obtain other functional indicators (e.g., glomerular permeability, renal tubular fluid, and blood flow) | Invasive; not able to detect global GFR; need more methodological validation | *           |

ROI, regions of interest. * : 0–10 peer-reviewed publications. ** : 51–200 peer-reviewed publications. *** : ≥200 peer-reviewed publications. The publications above were searched using the Scopus database (Elsevier).
Conclusion

As shown in Table 2, the GFR assessment methods in laboratory animals are more diverse than those in clinical practice. As endogenous markers are inaccurate, inulin is operationally complex, many exogenous markers have been developed to replace inulin, including iohexol, fluorescent tracers, radiolabeled markers, and unlabeled radiocontrast agents (Table 2). Among them, the FITC-sinistrin tGFR appears to have the most potential with over 150 peer-reviewed publications. However, further methodological validations, including the repeatability on the same individuals, should be performed, especially for obese animals. Additionally, FITC-sinistrin cannot detect the GFR of a single kidney.

Benefiting from in vivo high-resolution fluorescence microscopy technology, snGFR, and the entire dynamic process of glomerular filtration can simultaneously be acquired, thereby supplying more information related to renal function. However, the accuracy of this method needs to be further improved or validated.

Briefly, an ideal animal GFR assessment method should be accurate, safe, noninvasive, and can assess the GFR of a single kidney or nephron. Animals should be awake and unfettered during GFR measurement. An ideal method should not be affected by nonrenal factors such as changes in body fluid volume and body weight. It would be better if other physiological information could be obtained during GFR measurements. Nonmammals should also be considered when developing GFR assessment methods, although the physiological structure and function differ from that of mammals. We believe that more innovative GFR measurement methods will be developed in the future to empower nephrology research.

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Conflict of Interest Statement

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