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NEW CONCEPT

Optimization of a Technique to Standardize the Rodent Roux-En-Y Gastric Bypass Model and Troubleshooting of Postoperative Failures

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Abstract

Background Rodent models are required in studies on the mechanism of Roux-en-Y gastric bypass (RYGB). However, the construction of the model is hard, and there are various causes of death after surgery in rats.

Methods RYGB models with procedures containing a series of anatomic landmark were established in rats. Optimized procedures during surgery, possible complications after surgery, and corresponding solutions were studied.

Results With the introduction of perioperative nursing and optimized surgery procedures, less time-consuming surgeries were performed and higher survival rates were achieved. Trouble-shooting data based on death time points are listed and discussed for various causes of failure.

Conclusions This study provides practical suggestions for investigators to perform RYGB surgery on rats. The troubleshooting suggestions will help operators to efficiently identify problems in their procedures.

Keywords RYGB · Rodent model · Surgical procedure · Trouble shooting

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Introduction

Obesity has become a global health issue and an area of research focus [1]. Bariatric surgeries are effective therapies for obesity and related metabolic diseases. Roux-en-Y gastric bypass (RYGB) is one of the most commonly used bariatric surgeries [2, 3]. In vivo evidence such as changes in gut hormones and variation in protein and gene expression before and after surgery can only be acquired in animal models. Thus, a well-designed rodent RYGB model is very important and is urgently required.

In humans, the RYGB procedure consists of the creation of a small gastric pouch and the bypass of the proximal small bowel [2, 3]. Initially, the gastric pouch was designed as the restrictive component and the bypass as the malabsorptive part. A rat model of RYGB is widely used, as it can mimic the time profile and magnitude of human weight loss. Despite numerous differences between studies, the consensus in all reports is that [4–7]: (i) the construction of a small gastric pouch (2–20%), (ii) jejunum transection (10–40 cm) distal to the ligament of Treitz, (iii) the construction of the alimentary limb by gastro-jejunostomy, and (iv) the construction of the biliopancreatic limb by jejuno-jejunostomy are common between the different models.

The construction of a gastric pouch is technically demanding. A small pouch is better, but there seem to be higher chances of bleed, and this technique is time consuming [4]. To avoid the gastric vessels, a larger pouch was constructed; however, this resulted in insufficient weight loss or weight regain [8–11]. Some authors also used stapler devices in this part of the surgery [4, 12]. This is, of course, easier but makes the pouch too large. Another issue in this section is the vagal nerve. Total vagotomy in gastric bypass seemed to result in unsustained weight loss [13]. In contrast, by separating and ligating the left gastric vessels, the vagal fibers in the dorsal vagal trunk were preserved [6]. This could benefit the rats with sustained weight loss and better gut hormone pattern, although the mechanism is not clear yet.

There are considerable differences in the limb lengths in both clinical patients and rat models. In rat models, the alimentary limb length varied between 10 and 50 cm, and the biliopancreatic limb length ranged between 10 and 40 cm [4]. Initially, a longer alimentary limb was thought to achieve better caloric malabsorptive effects after surgery; however, the results remain controversial [14–16]. In fact, some researchers have argued that dietary fat content may be more important than limb length in terms of malabsorption [12, 17]. Gut hormones have also been shown to contribute to metabolic changes. Some studies have suggested that longer biliopancreatic and alimentary limbs could achieve augmented GLP-1 that could be beneficial for the metabolic outcomes of the surgery [18]. Decisions on limb lengths remain to be discussed, but as far as we are concerned, different lengths do not alter the difficulty of the surgery.

Mortality rates after gastric bypass are rarely reported and discussed, but they seem to range from 0 to 35% [4]. Stenosis and anastomotic leakages are the leading causes [7, 19, 20]. Problems usually occurred in the gastro-jejunal anastomosis area [7, 19, 20]. Differences in mortality rates were usually due to experience with the surgical procedure. However, very few indications for nonphysician scientists were suggested in these studies.

Besides these "big issues" directly affecting the short-term and long-term prognosis, there are many other small but important technical tips for performing the surgery. RYGB requires specific perioperative management, including temperature control, diet management, and liquid support. However, in most reports, this part was mentioned but not fully described and analyzed.

Different surgical designs lead to differences in the surgical outcomes. Although there is much to discuss in this area, we remain neutral in this debate, since there are similar arguments regarding clinical RYGB patients. Our aim is to shorten the surgery time and improve the survival rate, which is closely related to surgery skills and perioperative nursing. However, in previous studies, there are few comments on surgical details such as dissection, anastomosis, and troubleshooting. Thus, there is still no information for researchers who lack a surgical background when they face failure in constructing RYGB models. In our study, several explicit anatomical markers were used to direct the surgery and to minimize differences within the surgery groups. These markers restricted surgical variation and simplified the surgical instructions. An optimized procedure was recommended based on comparisons of different steps. Greater than 90% success rate is promised when following this protocol. Moreover, we categorized and classified the common causes of death according to different time periods. When death occurs, our trouble-shooting list will help researchers to quickly identify their mistakes and provide them with more clues to achieve a fast and consistently successful model.

Materials and Methods

Animals

Male Sprague-Dawley (SD) rats, 14 weeks old, were purchased from the Animal Breeding Center of Tongji Medical College, Huazhong University of Science and Technology. They were purchased in several cohorts to total 300 rats. Rats were housed in a specific pathogen-free (SPF) animal room (24 °C, 12-h light/12-h dark cycle, with lights on at 7:00 am). All animal studies, including the rat euthanasia procedure, were performed in compliance with the regulations and guidelines of Tongji Medicine College Institutional Animal Care Committee.

Quality Controls for the Model: Surgical Anatomic Landmarks

Various aspects of the surgery complicate the quality control of the model. Previous studies [4–7] and our experiences support the use of a series of markers for beginners to follow and locate during the surgical procedures:

1. Enter the abdominal cavity and identify the ligament of Treitz where the proximal jejunum fixes to the ileum (Fig. 1a).

(Step 1 should take 3–5 min).

2. Separate and severe the jejunum approximately 10 cm below the Treitz ligament [21, 22], and completely and firmly close the two cut ends by suture to avoid leakage after surgery (SILK 4-0). The ends are named the proximal jejunum (biliopancreatic limb) and distal jejunum (alimentary limb) according to their respective locations. (The lengths are determined by referring to the similar ratio in clinical RYGB surgery) (Fig. 1b).

(Step 2 may last 5–10 min)

3. Identify the ileocecal junction site and perform a proximal jejuno-ileostomy 25 cm from the site by performing side-to-side anastomosis using continuous sutures (PDS 6-0) [21, 22] (Fig. 1c).

(Step 3 may last 20 min)

4. Clean the tissue around the stomach, cutting off the gastro-hepatic and gastro-splenic ligaments to facilitate placing the stomach outside the abdominal cavity **(**Fig. 1d**)**. Open a 5-mm incision in the greater curvature of the stomach where there is low density of vascular networks and clean the inside of the stomach. Then, suture the incision and construct the stomach pouch (Fig. 1e).

Perform anastomosis between the stomach pouch and the distal jejunum (PDS 6-0) (Fig. 1f).
(Step 4 may last 30–40 min)

5. Use 37–40 °C warm saline to wash the abdominal cavity and double check for potential hemorrhage and intestine arrangements before closing the abdominal wall.

(Step 5 may last 5–10 min)

Perioperative Nursing

Preoperation:

1. At least 16 h of fasting is required. Oral rehydration salts are permitted during fasting. Rats should be singly housed in metabolism cages to avoid fighting and coprophagia.
2. Preheat the surgical table to 37 °C and keep it warm throughout the entire surgical process.

Immediately prior to surgery:

1. Anesthesia: 1% pentobarbital sodium (4 ml/kg, i.p.) is used for anesthesia. The anesthetic effect is maintained for 2–3 h.
2. Liquid supplement: 10 ml glucose and sodium chloride is administered via subcutaneous injection prior to surgery.

Post-operation:

1. The local temperature of the cage should be maintained at 35–37 °C overnight. Room temperature control should be maintained at approximately 34 °C for 2 days.
2. Subcutaneous injections of 10 ml glucose and sodium chloride are given twice daily for 3 days.
3. Intraperitoneal injections of penicillin (100,000 units/rat) and flunixin (1 mg/kg) are given once daily for 3 days for anti-inflammation and analgesia.
4. Postoperative fasting is maintained for 1 day. Glucose and saline are available 2 days after surgery. Boiled eggs are supplied 4 days after surgery. Normal diets are provided 1 week after surgery.

Techniques for Anastomosis

Generally, only one surgeon performs all operations on rat RYGB model. Therefore, a simple and easy procedure may become much more difficult. The following steps are recommended in Fig. 1g–h to make this procedure easy and smooth.

Continuous side-to-side anastomosis is recommended for the stomach-jejuno (Fig. 1h) and jejuno-ileum (Fig. 1g) anastomosis to avoid leakage, obstruction, or tearing of the mesentery due to excessive tension.

First, two traction sutures are used on both ends to fix the intestine. This step is very important if there is only one surgeon performing the surgery.

Second, the jejuno-ileum anastomosis: The inside part of the stoma is sutured first (Fig. 1g left), and the outside part (Fig. 1g right) is sutured second.

Third, the stomach-jejunum anastomosis: The inside part of the stoma is sutured first (Fig. 1h left), and the outside part (Fig. 1h right) is sutured second.

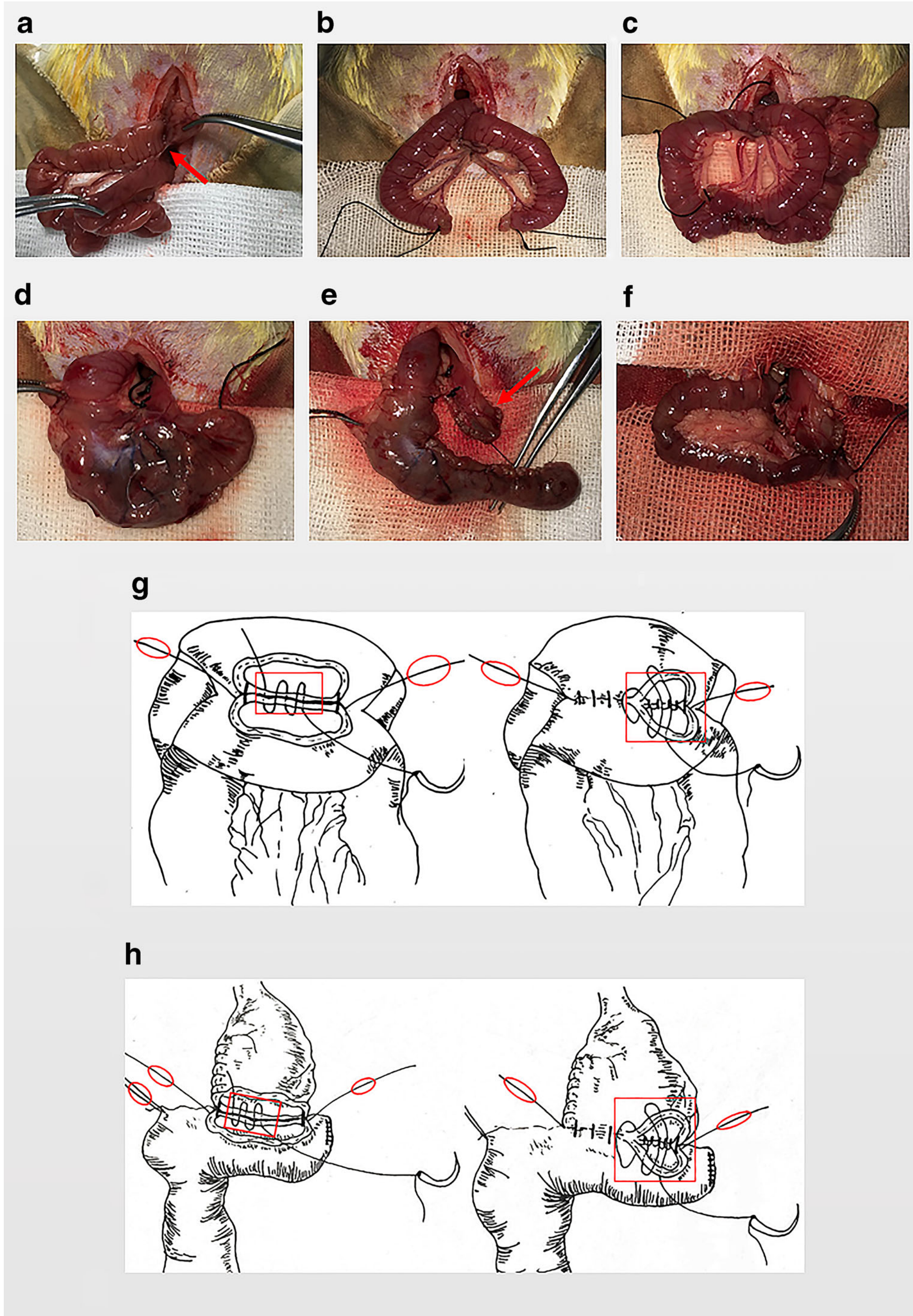

Fig. 1 **a** The ligament of Treitz. **b** Jejunum separation at a site approximately 10 cm from the Treitz ligament. **c** The jejuno-ileum anastomosis. **d** Isolation of the esophagus and fundus of the stomach. **e** Construction of the stomach pouch. **f** Anastomosis between the stomach pouch and jejunum. **g** Procedures for anastomosis: The two red ovals show the traction sutures used on both ends to fix the intestine. This step is very important in case there is only one surgeon performing the surgery. **h** Procedures for anastomosis: The two red ovals show the traction sutures used on both ends to fix the intestine. This step is very important in case there is only one surgeon performing the surgery

Construction of the Gastric Pouch

The abundant vascular network of the stomach makes the establishment of a gastric pouch the most difficult part of the surgery. In addition, the following tips may help (Fig. 1d, e):

First, approximately 5 mm below gastroesophageal junction is the dividing point between the gastric pouch and the remnant stomach. The volume of the pouch is approximately 5% of that of total stomach.

Second, the gastric pouch is clipped with a small vein clip and the remnant stomach is clipped with a large artery clamp. Maintain approximately 3 mm distance between the two clamps to facilitate the amputation and suture of the stomach. Short-term clamping of the gastric tissue can suppress bleeding and does not affect tissue activity or blood supply.

Third, the vascular network of the remnant stomach is more abundant than that of gastric pouch. After the gastric disconnection, the broken end of remnant stomach should be closed quickly by continuous suture to avoid the occurrence of ischemic necrosis (PDS 6-0).

Fourth, partially suture the broken end of gastric pouch to adjust the outlet to the appropriate size, and subsequent gastrojejunostomy (PDS 6-0).

Fifth, special attention should be paid to the protection of the gastroesophageal vein in the process of establishing the gastric pouch to avoid hemorrhage caused by injury.

Analysis of Body Temperature Changes After Anesthesia

To study the effect of anesthesia on body temperature, we designed the following studies. The rats were anesthetized. The operator then opened the abdomen and closed it 0.5, 1, 1.5, and 2 h later ($n = 10$ in each group). Anal temperatures were measured every 1 h after surgery.

Effects of Room Temperature on the Early Time Point Survival Rates

After RYGB surgery, the rats were put at different room temperatures (from 25 to 40 °C) for the first night after surgery ($n = 10$ in each group). Survival rates were calculated the next day.

Optimized Surgical Procedures: Surgical Sequence of Anastomosis

There are two key procedures in the surgery: the jejuno-ileum anastomosis and the stomach-jejunum anastomosis. Either anastomosis may be performed first. We performed both surgeries by the same person and compared survival rates and surgery time. There were 20 rats in each group. We then analyzed the duration time for each surgery and the survival rate.

Weight Loss Analysis of the Surgery

The weight of the rats after RYGB was measured every week. Postoperative %total weight loss (%TWL) in different groups were recorded ($n = 10$ in each group). The sham group was the group which only underwent opening and closing of the abdomen. The control group only received anesthesia.

Statistical Analysis

Data are shown as the mean ± standard error of the mean. Statistical significance was evaluated by the Mann–Whitney *U* test or by Student's paired *t* test. $p < 0.05$ was accepted as statistically significant. All analyses were performed using SPSS v 17.0 software (SPSS Inc., Chicago, IL).

Results

In our study, we found that control of the surgery duration is very important for the postsurgery prognosis. When the anesthesia lasts for more than 1 h, rats find it hard to maintain body temperature without an external warming system (SFig1 a). Additionally, we found that after surgery, maintaining the room temperature at approximately 35 °C significantly increase the survival rates (SFig1 b). Therefore, external thermal insulation should be used during and after RYGB to help the rats overcome this very early dangerous period after surgery.

By comparing how long each procedure type takes, we found that the group in which the jejuno-ileum anastomosis was performed first took shorter time (SFig1 c) and gave higher survival rates (SFig1 d). Thus, we recommend that surgeons complete the jejuno-ileum anastomosis first.

After RYGB surgery, there were significant weight-loss effects for all the HFD rats. However, some rats became extremely thin but remained alive for a long time. Upon autopsy, we found that those rats exhibiting sustained losses of body weight generally showed signs of incomplete obstruction. Thus, experimental data from these rats are not reliable. In fact, we found that, even after RYGB surgery, the decrease in body weight generally lasted for only 3–4 weeks. This regain of weight is normal (SFig1 e).

Trouble Shooting

There are various factors that could lead to death. For different operators, there are different tendencies. However, these factors could be classified according to the time points at which death occurred. Detailed description and troubleshooting strategies are listed in Table 1.

Discussion

There is a great variety of RYGB models. Nevertheless, the human phenotype, in terms of weight loss, has already been successfully mimicked in most rodent models. These rodent models all contain the following key components: a gastric pouch and the alimentary/biliopancreatic limb [4, 6]. Previous studies suggested that a larger pouch may be related to weight regain, and a small pouch may restrict the meal size rather than the total food intake [7, 19]. On the other hand, different alimentary lengths were also used in an attempt to investigate the malabsorptive mechanism. It has been suggested that a very long alimentary limb may be superior for type 2 diabetes [23]. However, these conclusions still remain controversial [14–16].

In the current study, we strictly follow the surgical approach used in patients. Many surgeons report that at least

Table 1 Complications and potential causes of death in different time points and solutions for them

Time point	Potential cause of death	Solution
Day 0 (< 12 h)	Hypothermia; overdose of anesthetic; serious hemorrhage (Fig. 2a)	Keep RT in 35–37 °C for 12 h after surgery; avoid disturbing blood vessels around the lesser curvature of stomach
Days 1–2	Low room temperature; serious anastomotic leakage (Fig. 2b); slow but consistent hemorrhage	Keep RT around 32–34 °C for the first 2 days after surgery; anastomosis skills are listed in (Fig. 1g, h); be aware of high risk of hemorrhage around the esophagogastric junction
Days 3–5	Infection (Fig. 2c); complete anastomotic obstruction (Fig. 2d); mild anastomotic leakage	Antibiotics required; do not suture too densely
Days 7–9	Infection; lack of nutrients; incomplete anastomotic obstruction in the gastrointestinal anastomosis area; complete anastomotic obstruction in the jejuno-ileum area; mild anastomotic leakage in the gastrointestinal anastomosis area	Antibiotics required; anastomosis skills are listed in (Fig. 1g, h); during surgery, gastrointestinal anastomosis should be done last
Days 10–14	Infection; incomplete anastomotic obstruction in the jejuno-ileum area; mild anastomotic leakage in the jejuno-ileum area	Antibiotics required; nutrient support if there is weight extremes; anastomosis technique is recommended in the article to avoid obstruction or leakage
Weeks2–4	Incomplete anastomotic obstruction in the jejuno-ileum area; anastomotic leakage due to bad blood supply (not due to surgical suture) in the local area	Fewer sutures are better than denser sutures in terms of avoiding bad blood supply in the anastomotic stoma
Weeks 4–8	Malnutrition; gastroesophageal reflux; unsuitable food supply (e.g., too hard to go through the anastomosis area)	Diet management: liquid-semiliquid-solid diet; additional subcutaneous injection of saline and glucose when oral pathway is not sufficient

90% of the stomach is bypassed and that only 1–2% of the stomach is left contiguous with the jejunum [24, 25]. Thus, in our study, we chose to construct a small gastric pouch, which better mimics the clinical situation. Although there are different opinions on length, we did not strictly recommend a set length because different lengths may have different therapeutic effects, which may be suitable for different patients. Instead, we marked all important anatomic landmarks for reference. These landmarks will help operators to further investigate their own models.

Limiting the duration of the surgery is an important principle to increase the success of the surgical model. In our study, we compared the sequences of two places for anastomosis; the stomach-jejuno and the jejuno-ileum. In many reports, the stomach-jejuno anastomosis was done first [26–28]. However, in our study, we found that performing the jejuno-ileum anastomotic first takes a shorter amount of time, leading to a higher survival rate (SFig 1 c, d). In the surgery, the stomach-jejunum anastomosis is the most difficult part, so the higher chances of accidents during this step may affect the subsequent steps. For example, when there is hemorrhage, the surgical exposure would be very bad for the following steps. Another important factor is that the site of the jejuno-ileum anastomosis is actually the key point for the quality of the surgery. The site determines the length of the biliopancreatic limb and the alimentary limb. Therefore, a clear and unaffected abdominal environment is required. Since the stomach-jejunum anastomosis is the largest trauma during the whole surgery, we suggest performing this step last so that resuscitation can be conducted soon after this trauma.

Another key point of RYGB rodent model is excellent repeatability. Some operators have had excellent success rates. However, the weight-loss effects vary greatly. In our experience, different operators make different choices at many steps when performing the surgery. A lack of landmarks may have led to different destinations. Thus, in using our clinical experience, we have set a series of anatomic landmarks for reference during surgery. Strictly following these markers is very important for reducing the variability. Additionally, when there is a landmark, it will take less time in making decisions.

Anastomosis is the most important, yet most difficult, surgical technique. Interestingly, we found that many beginners tended to suture too densely, resulting in subsequent obstruction. In fact, we considered that slight leakage is better than incomplete obstruction. This is because incomplete obstruction cannot be self-cured. Rats with incomplete obstruction suffered from malnutrition and showed extremely high and unreliable weight-loss. In contrast, we found that the rodent experimental animals had a strong immune system, and in fact, slight leakage could almost always be quickly self-healed. The rats also started to regain body weight 4 weeks after RYGB surgery. Taking all these factors into consideration, we recommend operators to not suture too densely when performing anastomosis. Our principle is that loose is better than dense.

Systemic-supporting therapies in the perioperative period are vaguely described in many research articles, despite these factors being very important and heavily contributing to cases of postoperative death, especially for beginners. In our study, we found that during laparotomy, the maintenance of

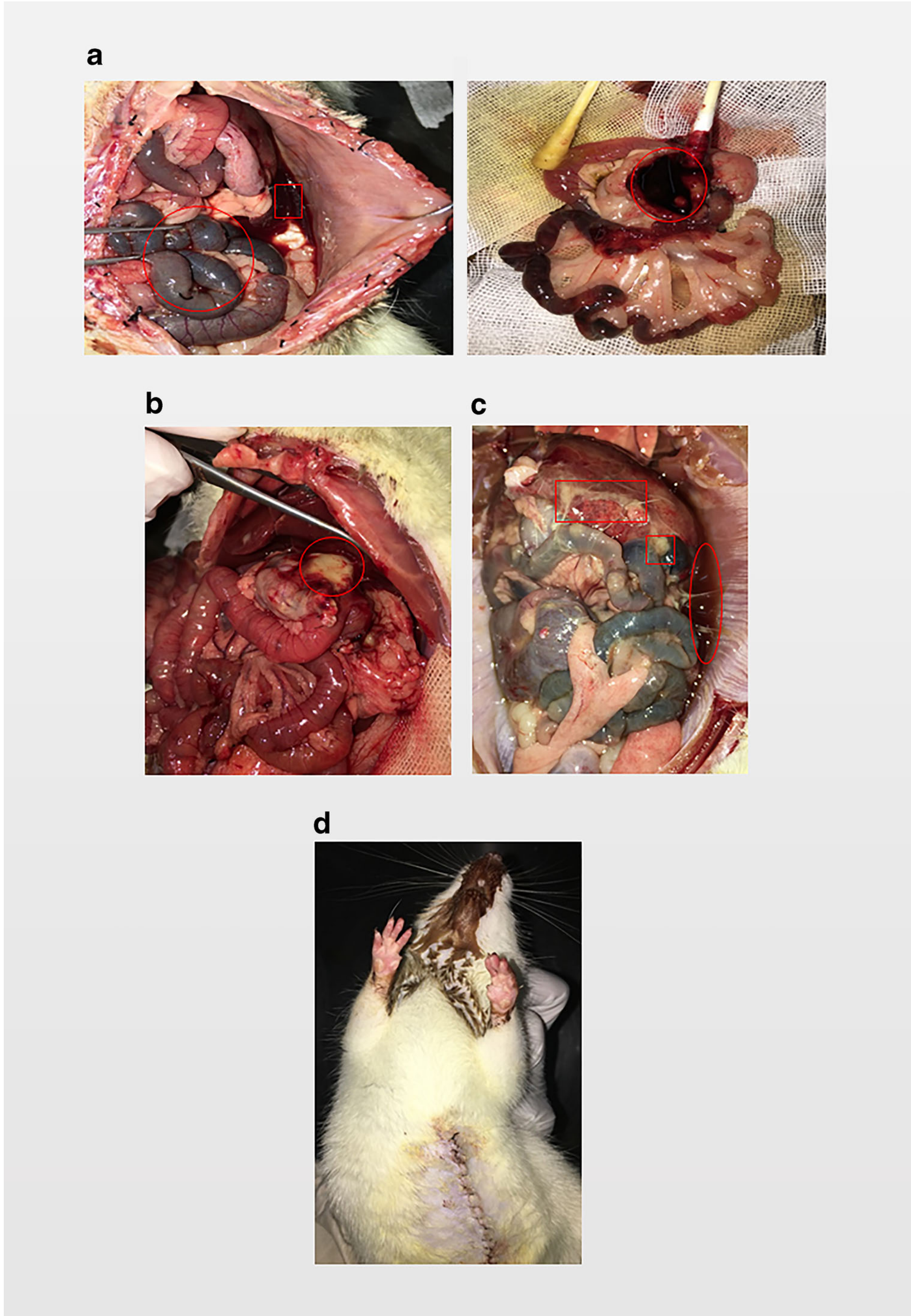

Fig. 2 **a** Hemorrhage after surgery. Bleeding was observed in abdominal cavity (marked in red square box) and within the lumen and stomach (marked in the red circle). **b** Anastomotic leakage after surgery. Fibrous capsule formation was identified around the leakage sites (marked in the red circle). **c** Infection inside the abdominal cavity (death occurred 5 days postsurgery): ascites (marked in the red circle) and empyema (marked in red square box) were observed. **d** Gastroesophageal reflux after surgery

anesthesia over 1 h resulted in postoperative hypothermia. However, even for a very skilled operator, 1 h is not sufficient for the surgery. Thus, temperature preservation is vital for the survival of rats after surgery. In addition, liquid support is also important for recovery from anesthesia. By adding subcutaneous injection pre- and post-operation, we significantly increased our success rate.

Although many previous studies have already introduced rodent methods of RYGB model, inexperienced researchers still meet with a high mortality rate when developing RYGB models in rats. For researchers, the failure of the surgery seems to be unavoidable in the beginning. However, it is difficult to improve because when death occurs, there are too many possible reasons. Different people may make different mistakes, making troubleshooting difficult for the individual. Thus, unlike previous reports that have focused on pouch/limb designs, our research tried to figure out a reference list for the most common causes of death. For example, some sutures are too tight, while others are too loose; some researchers may neglect the temperature; and some may arrange the food intake after surgery in the wrong way. However, the time point of death caused by certain problems, such as obstruction or leakage, is relatively steady. Thus, possible causes of death could be predicted by referring to the time table. This is especially valuable when the abdominal cavity is in a mess and autopsy failed to identify clear clues.

In conclusion, we explored the surgical techniques of RYGB in detail. We have identified anatomical markers to reduce the variability in the operative method. We have listed the key points and difficulties in the operation, have given the corresponding solutions, and have proposed a perioperative nursing plan. It is helpful for beginners to understand surgical skills, establish adjustable principles, and optimize operation details. While the operators practice in order to gather experience in the technique, this study helps them to quickly identify their own problems. In this study, we tried to help researchers to enhance their success rate more quickly, thus promoting further study of this interesting type of surgery.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

A Statement of Animal Rights/Ethical Approval The Ethics Committee for Animal Research of Tongji Medicine College approved all procedures in this study.

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