Challenges and Pitfalls of Experimental Bariatric Procedures in Rats

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Key Words
Animal models · Bariatric surgery · Surgery

Abstract

Introduction: The impact of Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) on obesity and obesity-related diseases is unquestionable. Up to now, the technical descriptions of these techniques in animals/rats have not been very comprehensive. Methods: For SG and RYGB, operating time, learning curve, and intraoperative mortality in relation to weight of the rat and type of anesthesia were recorded. Furthermore, a review of the literature on experimental approaches towards SG and RYGB in rats was carried out, merging in a detailed technical description for both procedures. Results: The data presented here revealed that the mean operating time for SG (69.4 ± 22.2 min (SD)) was shorter than for RYGB (123.0 ± 20.7 min). There is a learning curve for both procedures, resulting in a reduced operating time of up to 60% in SG and 35% in RYGB (p < 0.05; t-test). However, with increased weight, operating time increases to about 80 min for SG and about 120 min for RYGB. Obese rats have an increased intraoperative mortality rate of up to 50%. After gaseous anesthesia the mortality can be even higher. The literature search revealed 40 papers dealing with SG and RYGB in rats. 18 articles (45%) contained neither photographs nor illustrations; 14 articles (35%) did not mention the applied type of anesthesia. The mortality rate was described in 15 papers (37.5%). Conclusion: Experimental obesity surgery in rats is challenging. Because of the high mortality in obese rats operated under gaseous anesthesia, exercises to establish the techniques should be performed in small rats using intraperitoneal anesthesia.

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Introduction

Obesity and obesity-related diseases are an increasing health care problem within western countries and have become a leading cause of morbidity and mortality [1–3]. Obesity is the main contributory factor to the metabolic syndrome, encompassing dyslipidemia, essential hypertension, insulin resistance, and even insulin-dependent diabetes mellitus [4–6].

The positive impact of surgical interventions such as Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) in the management of obesity is unquestioned [1, 7–10]. Nowadays, bariatric procedures such as RYGB are used to treat type 2 diabetes mellitus not only in obese patients but also in non-obese insulin-dependent diabetic patients [11–15]. However, the underlying mechanisms responsible for these effects are not well understood [16–19]. This suggests that the number of experimental studies examining these issues will increase over the next few years – particularly as there are rodent models available for studying the effects of bariatric procedures on weight loss and/or the metabolic syndrome [20–24].

Despite well-written descriptions of bariatric procedures in animals presented in previously published literature, surgical details, comprehensive images, technical difficulties, and pitfalls are rarely described [20, 23–29]. This makes it difficult to figure out the intricacies of these models. Thus, the aim of this study is to present a detailed surgical description of SG and RYGB in rats, including technical details and advice on how to avoid potential pitfalls.

Material and Methods

Using PubMed, an online search for surgical techniques used in bariatric procedures in rat models was carried out. As mesh terms, the following words were used in different combinations: rat, model, bypass, Roux-en-Y bypass, sleeve, sleeve gastrectomy, bariatric surgery, obesity surgery. After reading the abstracts from 413 articles, 40 papers were thoroughly evaluated. From these 40 articles, methods of anesthesia, sutures/instruments, existence of photos/drawings, mortality rate, and placement of gastrojejunostomy (for RYBG) were extracted. The remaining 373 publications dealt with other procedures such as gastric banding, biliopancreatic diversion, or ileal transposition.

In preparation of an upcoming animal study on diabetic rats, the use of 60 ‘regular’ Sprague Dawley rats (Charles River) was approved by the German Regional Council in order to practice SG and RYGB. For each intervention (SG and RYGB), the weight of the rat, operating time, type of anesthesia, and intraoperative mortality were recorded. In addition, a detailed technical description of each procedure was given. Because of the retrospective nature of this study (surgical procedures, application of narcotic agents, and the weight of the rat were not prospectively planned), statistical analysis was only applied within SG or RYGB. Student’s t-test was applied to detect possible differences between groups.

Results

Literature Review

From 413 reviewed abstracts, the literature searches eventually uncovered 40 papers dealing with SG or RYGB (10 papers described SG, 30 papers related to RYGB (table 1 and table 2)). All papers were scientifically well written with interesting findings. However, from a technical point of view, the content of most of the papers was unsatisfactory. That is, even though the written technical description was often sufficient, graphical depiction of the surgical procedures was lacking. In 18 papers (45%) neither photographs nor drawings were
shown. Based on our subjective assessment, only 5 papers (12.5%) contained sufficient images of the surgical techniques. Concerning the use of narcotic agents, intramuscular injection, intraperitoneal (i.p.) application, and gaseous anesthesia were used. 14 articles (35%) did not mention the method of applied anesthesia. The mortality rate was described in 15 papers (37.5%). In these 15 papers, the mortality rate ranged from 0% to 42%.

**Experimental Data**

**Preparation of Animals**

All animals had free access to tap water (also during fasting time) and were fed standard rat food pellets. Rats undergoing surgery were fasted for at least 12 h before surgery. Initially, it was strongly recommended by our animal facility to fast rats just for about 6 h before surgery. However, with only 6 h of fasting, rats still had a lot of food within the stomach, which had to be removed at the beginning of the surgical intervention. Subsequently, contamination of the surrounding tissue was unavoidable.

**Preparation of Operating Field**

Rats were placed on a heat plate, which was adjusted to 38°C to prevent heat loss during surgery. A 10-ml injection syringe filled with saline was placed on the plate for warm saline solution supply during the experiment. Aseptic pledges, four adhesive strips and the operating instruments were prepared on a sterile towel. After realizing that special instruments for small animals were not suitable, we changed to regular surgical instruments used in pediatric surgery. To take precautions against bleeding, bipolar cautery was used.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Anesthesia</th>
<th>Sutures</th>
<th>Instruments</th>
<th>Images shown</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>[21]</td>
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<td>not described</td>
<td>not described</td>
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<td>not described</td>
<td>no</td>
<td>n/a</td>
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<td>[32]</td>
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<td>5-0 polypropylene</td>
<td>bulldog forceps</td>
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<td>[33]</td>
<td>sodium pentobarbital, i.p.</td>
<td>000-gauge thread</td>
<td>atraumatic hemostatic forceps</td>
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<td>35%</td>
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<td>staple line, 6-0 polyglycolic acid</td>
<td>Stapler TX 30 B-Ethicon</td>
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<td>0%</td>
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<tr>
<td>[34]</td>
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<tr>
<td>[35]</td>
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<td>3-0 Silk</td>
<td>tonsil clamp, scalpel</td>
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<tr>
<td>[36]</td>
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<td>cautery, blade retractor</td>
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<td>0%</td>
</tr>
<tr>
<td>[36]</td>
<td>chloral hydrate, i.p.</td>
<td>8-0, non-absorbable</td>
<td>cautery, blade retractor</td>
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<td>n/a</td>
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<tr>
<td>[29]</td>
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<td>non-absorbable</td>
<td>not described</td>
<td>Yes</td>
<td>n/a</td>
</tr>
</tbody>
</table>
**Table 2.** Technical details of Roux-en-Y gastric bypass as described in reviewed the literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Anesthesia</th>
<th>Visceral sutures</th>
<th>Instruments</th>
<th>Images shown</th>
<th>Placement of gastrojejunostomy</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>[24]</td>
<td>ketamine/xyalazine, i.p.</td>
<td>titanium staple line, 4-0 &amp; 5-0 polyglactin</td>
<td>Stapler TRH30-4.8/Ethicon, Metzenbaum scissors</td>
<td>yes</td>
<td>end-to-side separate anastomose</td>
<td>4%</td>
</tr>
<tr>
<td>[37]</td>
<td>ketamine/xyalazine, i.m.</td>
<td>titanium staple line, 4-0 &amp; 5-0 polyglactin</td>
<td>Stapler TRH30-4.8/Ethicon</td>
<td>yes</td>
<td>end-to-side separate anastomose</td>
<td>37%</td>
</tr>
<tr>
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<td>not described</td>
<td>not described</td>
<td>no</td>
<td>not described</td>
<td>n/a</td>
</tr>
<tr>
<td>[38]</td>
<td>ketamine/xyalazine, i.p.</td>
<td>titanium staple line, 5-0 polyglactin</td>
<td>Stapler TRH30-4.8/Ethicon</td>
<td>yes</td>
<td>end-to-side separate anastomose</td>
<td>n/a</td>
</tr>
<tr>
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<td>n/a</td>
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<tr>
<td>[41]</td>
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<tr>
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<td>end-to-side separate anastomose</td>
<td>n/a</td>
</tr>
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<td>[46]</td>
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<td>6-0 running suture</td>
<td>not described</td>
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<td>end-to-side separate anastomose</td>
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<tr>
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<td>Not described</td>
<td>n/a</td>
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<td>[48]</td>
<td>ketamine/atropine/ diazepam</td>
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<td>no</td>
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<td>n/a</td>
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<td>[26]</td>
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<td>staple line, 5-0 vicryl and 5-0 prolene</td>
<td>US surgical stapler (US Surg. EndoGIA 60x2.5mm)</td>
<td>yes</td>
<td>not described</td>
<td>n/a</td>
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<tr>
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<td>not described</td>
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<td>not described</td>
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<tr>
<td>[22]</td>
<td>isoflurane</td>
<td>staple line, 5-0 silk-sutures</td>
<td>GIA stapler ETS-Flex/Ethicon</td>
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<td>n/a</td>
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<tr>
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<td>5-0 polypropylene</td>
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<td>yes</td>
<td>not described</td>
<td>23%</td>
</tr>
</tbody>
</table>

*Table 2 continued on next page*
**Anesthesia**

Owing to experience, the first animals were anesthetized with an i.p. injection of xylazine (5 mg/kg, 2% solution) + ketamine (100 mg/kg 10% solution). However, since animals weighing more than 300 g are difficult to handle and one of the prerequisites at our animal facility was that two persons had to be present in order to administer i.p. injections in conscious rats, we changed to gaseous anesthesia (initially 5% isoflurane mixed with 1,000 ml oxygen, for maintenance 3% isoflurane mixed with 750 ml oxygen). Unfortunately, under gaseous anesthesia there was an intraoperative mortality rate of 100%. Despite being very cautious with the anesthesia, this mortality rate did not drop down. Therefore, we switched to the combination of gaseous anesthesia (5% isoflurane mixed with 1,000 ml oxygen) followed by an i.p. injection of xylazine (5 mg/kg, 2% solution) + ketamine (100 mg/kg, 10% solution) every 30 min for maintaining anesthesia. Using this approach, we were able to reduce the mortality rate to 0% for SG and 25% for RYGB, even in obese rats weighing more than 400 g.

**Table 2** (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Anesthesia</th>
<th>Visceral sutures</th>
<th>Instruments</th>
<th>Images shown</th>
<th>Placement of gastrojejunostomy</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>[52]</td>
<td>isoflurane</td>
<td>5-0 silk</td>
<td>GIA Stapler ETS-Flex/Ethicon</td>
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<td>not described</td>
<td>n/a</td>
</tr>
<tr>
<td>[53]</td>
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<td>staple line</td>
<td>Stapler/ Ethicon Endo-Surgery</td>
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<td>end-to-side separate anastomose</td>
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<td>[54]</td>
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<td>not described</td>
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<tr>
<td>[55]</td>
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<td>not described</td>
<td>not described</td>
<td>n/a</td>
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<td>not described</td>
<td>not described</td>
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<td>not described</td>
<td>n/a</td>
</tr>
<tr>
<td>[56]</td>
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<td>not described</td>
<td>not described</td>
<td>yes</td>
<td>not described</td>
<td>n/a</td>
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<tr>
<td>[57]</td>
<td>ketamine/xylazine, i.p. 6-0 polypropylene</td>
<td>not described</td>
<td>not described</td>
<td>no</td>
<td>end-to-side separate anastomose</td>
<td>0%</td>
</tr>
<tr>
<td>[58]</td>
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<td>staple line</td>
<td>Stapler ATW35/Ethicon</td>
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<td>end-to-side separate anastomose</td>
<td>40%</td>
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<tr>
<td>[59]</td>
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<td>not described</td>
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<td>Cutting Stapler ATW35; Ethicon Endo-Surgery Inc.</td>
<td>no</td>
<td>not described</td>
<td>20%</td>
</tr>
</tbody>
</table>
General Setting

After anesthesia, the lower limbs of the rats were fixed with adhesive strips. The abdomen was shaved and disinfected with alcohol. Starting just below the xiphoid process a 3-cm midline incision was made with straight scissors. In order to keep the abdomen open, one stitch was placed on either side of the incision and fixed by adhesive strips.

After laparotomy, i.p. antibiotics (metronidazol 1.5 mg / 100 g, cefotaxime 3 mg / 100 g) were administered. The stomach was identified and carefully exposed. It was important to be very gentle in order to preserve the spleen and pancreas from iatrogenic injuries. To avoid maldigestion, both parts of the stomach (proximal stomach = fore stomach, distal stomach = glandular stomach) needed to be preserved for both SG and RYGB.

During the procedure, 10–20 ml/kg/h saline were injected every 30 min to prevent dehydration. At the end of the procedures, abdominal lavage was performed using 10 ml preheated saline (37 °C). For all anastomosis and intraperitoneal sutures, non-resorbable 5-0 or 6-0 prolene suture (Ethicon, Norderstedt, Germany) with a C-1 needle were used. To ensure open anastomosis and/or detect possible insufficiencies of anastomosis or sutures, saline was injected into the lumen of the respective intestinal structures. In case of suspected insufficiency, this part was closed by a z-suture.

The fascial layer was closed with a non-absorbable running 4-0 vicryl suture (Ethicon). The skin was closed using absorbable interrupted 4-0 prolene suture (Ethicon).

SG

The great omentum was carefully divided along the great curvature of the stomach. In order to empty the stomach from remaining food, a small incision was made at the greater curvature. To ensure the same size of the gastric sleeve, a regular infusion line (12.1 Ch) was shortened and placed inside the stomach along the smaller curvature. The stomach was cut along this infusion tube. The incision line for the SG is shown in figure 1.
However, it appeared that after cutting the stomach wall, the mucosa furled tremendously, making it very difficult to suture the sleeve adequately. Thus, 2–3 single stitches were placed along the resection line (Fig. 2). These 2–3 stitches had two functions. Firstly, they prevented the mucosa from furling, making the final running suture very easy. Secondly, they also functioned as anchor points for the running suture. That is, the free thread ends of each of the single stitches were tied to the running suture that was used to close the stomach in order to form the gastric sleeve.

**RYGB**

To empty the stomach, an incision was made at the greater curvature, 1 cm below the limiting ridge between the fore stomach and glandular stomach. After emptying the stomach, cautery and scissors followed the incision line to the middle of the smaller curvature (as shown in Fig. 1).

Meticulous preparation and thorough control of possible bleeding is vital, especially at the smaller curvature. If bleeding occurs, it is generally fatal for the animal. Ligature of the vessels at the smaller curvature was not performed.

The distal part of the stomach was now closed following the same procedure as described for the SG, starting with 2 single stitches to adapt the anterior and posterior walls of the stomach. Then the distal part was closed by a running suture, using the 2 single stitches as anchor points. The proximal part of the stomach was also adapted by 2 stitches and was closed starting by using a running suture at the greater curvature until the first single stitch was reached. After reaching this point, the jejunum was divided 10 cm distal of the ligament of Treitz. The distal end of the jejunum was pulled up to the stomach, anterior to the colon. To avoid contamination, the opening of the proximal part of the jejunum was placed into a sterile gauze outside the abdomen.

The dorsal wall of the gastrojejunostomy was included in the running suture that closed the proximal part of the stomach (Fig. 3a). After finishing the dorsal part of the gastrojejunostomy, the remaining proximal part of the stomach was closed using the single stitch as anchor point. The front wall of the gastrojejunostomy was closed by 4–5 single stitches (Fig. 3b).
Jejunojejunostomy

The proximal end of the jejunum needs to be connected end-to-side to the jejunum about 15 cm distal to the gastrojejunostomy by 2 running sutures. Each of the corners of the anastomosis was approximated with 1 suture. Using these sutures, the dorsal wall was closed; 3 stitches were generally sufficient. Both sutures were knotted (three knots), and the ‘other’ running suture was used to close the anterior wall of the anastomosis. Again, both sutures were knotted together.

Surgical Results

The main results are shown in figure 4. In general, SG (69.4 ± 22.2 min (mean ± SD)) was performed faster than RYGB (123.0 ± 20.7 min) (p < 0.001). There is a learning curve when operating on small animals. The operating time for SG and RYGB improved from Group 1 (SG: 106.7 ± 15.3 min; RYGB: 150 ± 3.2 min) to Group 3 (SG: 39.4 ± 7.5 min (p < 0.01); RYGB: 101.3 ± 17.7 min (p < 0.05)). However, the heavier the animal, the longer it took to finish the procedures. Even with expertise, the operating time on obese rats weighing more than 300 g was about 80 min for SG and about 120 min for RYGB. However, comparing operating times of animals using 300 g as threshold, the differences are not statistically significant (p > 0.5).
Based on our experience, the mortality in these procedures can be quite high. In the beginning, the mortality rate was up to 100% (Group 1). With more experience, mortality decreased to almost 0% (Group 3). However, the heavier the animal, the higher the expected mortality. Analysis of all animals (independent of experience and application of narcotics) revealed mortality rates of 30% in rats weighing up to 300 g whereas rats weighing more than 300 g had a mortality rate of 50% (data not shown). The method of narcotic administration had a tremendous impact on mortality. Using exclusively gaseous anesthesia, all animals died during the surgical procedure, which was not the case with i.p. application. As a consequence, a mixture of gaseous anesthesia and i.p. application was used. However, it is reasonable to suggest that a certain intraoperative mortality rate will remain if using animals weighing over 300 g.

**Discussion**

Even though the positive effects of bariatric procedures on weight loss and metabolic syndrome in humans are known, the underlying mechanisms responsible for these effects are not well understood. Thus, it seems reasonable to assume that the number of experimental animal studies analyzing these mechanisms will increase over the next few years. A review of the pertinent literature revealed that the technical descriptions of surgical tech-
niques are often well written. However, other important details, such as the method of anesthesia, illustrations and mortality rate, are not very detailed, and potential pitfalls are rarely described. This was the main reason for us to present the results of our initial experiences of SG and RYGB in rats.

Based on the data presented here, two main conclusions can be drawn. Firstly, intraoperative mortality and operating time strongly correlates with increasing weight of the animal. Secondly, anesthesia performed purely with gas seemingly increases intraoperative mortality.

One pitfall of this study is the statistical analysis, i.e. only time differences within the two groups (SG, RYGB) but no analysis of differences between SG and RYGB was performed. The main reason for this is that there was no prospective study plan. The results described here often emerged in parallel. For instance, the first operations were SG, performed on about 12 rats weighing less than 300 g. After that RYGB on rats with the same weight was done. However, experiments with SG in more obese rats were started simultaneously. To give another example, we only performed gaseous anesthesia in rats with RYGB. The technique was not tested on rats with SG because of the disastrous RYGB results. This makes it difficult to apply statistics between the groups (operating time, mortality, etc.).

However, the finding that gaseous anesthesia led to an intraoperative mortality rate of 100% in rats weighing more than 300 g is of some importance. We do not have a clear explanation for this pattern, but based on previous publications it can be assumed that accumulation of narcotic agents in the fat tissue combined with surgical stress might be responsible [30]. We tried to decrease the mortality rate by different approaches, such as applying as little gas as possible and asking for external support. However, we did not succeed. It may be that in experienced hands, gaseous anesthesia is as good as i.p. anesthesia. However, one recommendation resulting from this study would be that researchers who are not familiar with gaseous anesthesia should use i.p. anesthesia or a combination of gaseous and i.p. anesthesia.

Concerning technical details, some authors suggest placing the gastrojejunostomy on the anterior surface of the gastric wall, similar to humans, creating a complete separate anastomosis. We tested this approach several times. However, it was technically demanding and time-consuming. Thus, we tried to include the gastrojejunostomy into the running suture that closes the proximal part of the stomach. This technique worked quite well. It saved time, and so far there were no insufficient anastomoses. Furthermore, placing single stitches whenever running sutures were applied was also useful. The mucosa of the rat stomach vastly furled after cutting. The single stitches not only prevented this furling but also supported the running suture as abutment, making the anastomosis quite safe.

**Conclusion**

Experimental obesity surgery in rats is challenging and needs practice. Initial exercises should be performed on rats weighing less than 300 g, using i.p. anesthesia. However, a certain degree of intraoperative mortality is still to be expected, which should be taken into account when calculating the number of animals required.

**Acknowledgements**

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Disclosure Statement

There are no conflict of interests to state.

References


