

# Do iatrogenic serosal injuries result in small bowel perforation in a rabbit model?

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**Background and objectives:** Surgical dogma dictates that serosal injuries should be repaired during laparotomy as these injuries may result in localised areas of bowel ischaemia and may perforate. No study has investigated whether there is a correlation between the extent of serosal injuries and the risk for perforation under normal physiological conditions. We hypothesized that small bowel serosal injuries do not result in early or late perforation at physiological intraluminal pressures regardless of their size.

**Methods:** An in-vivo rabbit small bowel serosal injury model was developed and two experiments were conducted. The first – to determine whether and at which pressures various lengths and circumferences of serosal injuries in small bowel result in immediate bowel perforation – was performed infusing saline into isolated bowel segments with or without a variety of serosal injuries. In the second study – to determine whether or not serosal injuries result in delayed perforation – a range of injuries was created in rabbits and the effect assessed at re-laparotomy 5 days after the creation of the injury.

**Results:** No perforations were observed at the site of serosal injuries at physiological intraluminal pressures. Perforations occurred at  $43.7 \pm 18.6$  cmH<sub>2</sub>O,  $23.3 \pm 14.4$  cmH<sub>2</sub>O, and  $24.4 \pm 23.9$  cmH<sub>2</sub>O for controls, 4 cm long and 100% circumference serosal injuries respectively (p-value = 0.18 for various lengths and 0.71 for various circumferences). No serosal injuries perforated within 72 or 120 hours after creation.

**Conclusion:** Small bowel serosal injuries do not perforate or leak at physiological intraluminal pressures, either at the time of creation or up to 120 hours thereafter.

**Keywords:** serosal injury, laparotomy, perforation.

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## Introduction

A common dilemma faced by surgeons during laparotomy is whether or not to repair isolated small bowel serosal injuries. Surgical dogma dictates that serosal injuries should be repaired as not doing so may result in a weakened bowel wall as a result of absent anatomical support of the serosal layer with or without a localised area of ischaemia which may perforate.<sup>1</sup> Resulting intestinal leakage or fistula formation carries a potentially significant morbidity.<sup>2</sup> However, it is not known whether serosal injuries truly weaken the bowel wall or whether the blood supply of the mucosal and submucosal layers is adversely affected and whether this would predispose the small bowel to perforation at physiological intraluminal bowel pressures. According to the study, Intubation Study Of The Human Small Intestine, conducted by W.O. Abbott in 1942, the normal physiological pressure of 8 to 10 cm water is present in the human small intestine.<sup>3</sup>

Branches of mesenteric arteries penetrate the serosal and muscular layers of the bowel wall terminating in a

submucosal plexus, supplying the bowel in a radial fashion.<sup>4</sup> Bowel perforation is assumed to occur at the site of a serosal injury through combinations of local devascularisation and peristalsis-related increase in intraluminal pressure decompressing through the weakened bowel wall.<sup>1</sup>

We hypothesized that small bowel serosal injuries of any length or circumference would not perforate at physiological intraluminal pressures, either at the time of injury or during the first five postoperative days.

## Material and methods

Two experiments were conducted using the same serosal injury model. In the first experiment, 12 adult New Zealand White rabbits weighing between 3500 g and 4500 g were used. Midline laparotomies were performed under general anaesthesia, induced with intramuscular ketamine 100 mg/kg, and maintained with xylazine 5 mg/kg. Analgesia was administered intraoperatively every 2 hours with

subcutaneous buprenorphine 0.05mg/kg. A surgeon wearing 2.5 x surgical loupes using a size 11 scalpel created serosal injuries, either as a longitudinal incision or circumferentially. For longitudinal serosal injuries, a linear tear was created along the antimesenteric border of the small bowel ranging from 1 cm to 4 cm in length. For circumferential injuries the serosa between parallel incisions 1 cm apart were removed for varying percentages of the circumference ranging from 25%, 50%, 75% to 100% respectively as depicted in Figure 1.

The segment of bowel was clamped using non-crushing bowel clamps 5 cm away on either side of the serosal injury to ensure that the vascular supply of the isolated bowel segment was not compromised. Two 18-gauge peripheral intravenous catheter needles were inserted into the isolated bowel segment's lumen. A purse string suture using 6.0 PDS was stitched at the puncture site of the needles to prevent leakage of fluid from the small bowel. One intravenous catheter was used to incrementally infuse normal saline into the lumen until perforation occurred; the second intravenous catheter was connected to a manometer and used to measure intraluminal pressure generated by the infused normal saline (Figure 2).<sup>5</sup> To minimize the number of animals used, several isolated sections of small bowel were tested in each animal. On average, 9 segments were isolated from each animal (ranging from 6 to 16 segments due to the various length of small bowel in different animals). The entire length of small bowel distal to the ligament of Trietz was isolated. A sham operated control animal was included in each experiment to measure the pressure required to perforate a normal segment of bowel without serosal injuries. On completion of each experiment, before the animals were euthanized, the length of small bowel was harvested and placed in 10% formalin. All specimens were sent to the IDEXX laboratory (Pty) Ltd from the Faculty of Veterinary Science in University of Pretoria and the specimens were examined histologically to confirm that the injury only involved the serosal layer and to assess whether or not ischaemia was present at the perforation site.

In the second experiment, 10 adult New Zealand White rabbits were used. A single 4 cm long 100% circumference serosal injury was performed in each rabbit as described earlier without bowel clamping. At re-laparotomy, performed after 72 hours in 5 rabbits and after 120 hours in five, the injured small bowel segments were harvested and examined macroscopically as well as histologically for signs of bowel perforation. The animals were then euthanized. For the duration of the experiments animals were kept in the laboratory of Central Animal Service and nursed by the surgeon and nursing staff with 6 hourly clinical observation for signs of abdominal distension or peritonitis.

Figure 1. Illustration of various serosal injuries created in the small bowel. (a) a serosal injury 4 cm long involving < 1% circumference, (b) a serosal injury 1 cm wide involving 25% of the circumference, (c) a serosal injury 1 cm wide involving 100% of the circumference.

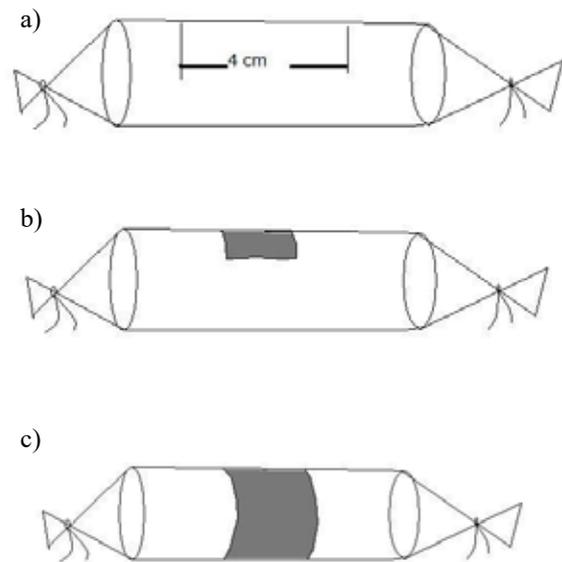
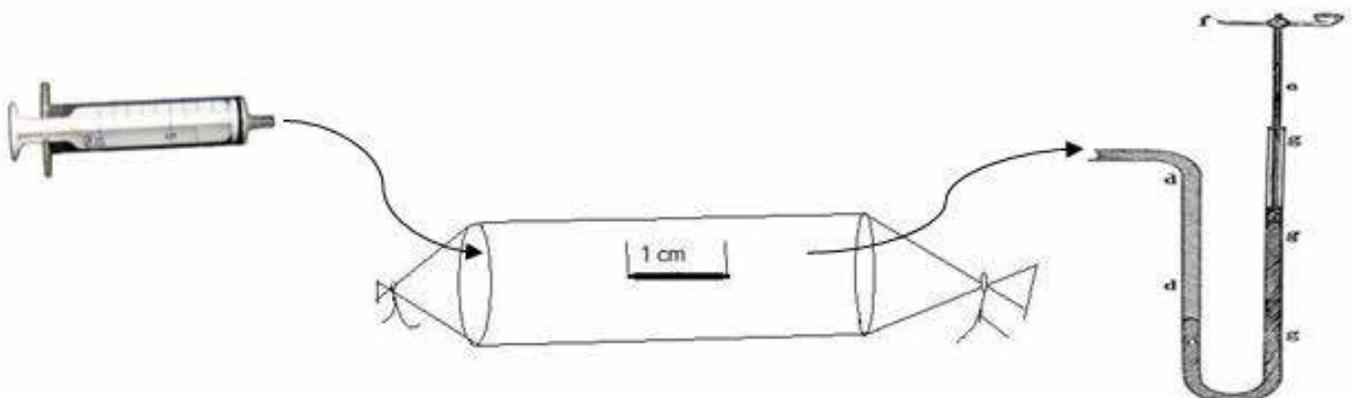


Figure 2. Rabbit small bowel serosal injury model. Experimental apparatus and set-up illustrating clamped off bowel section with a 1 cm long longitudinal injury of < 1% of the circumference. Intraluminal pressure was increased by infusing normal saline and pressure changes were monitored using a water manometer shown on the right side of the figure.



**Table 1. Perforation pressure of serosal injuries according to the longitudinal (in cm H<sub>2</sub>O) or circumferential (in % of the bowel circumference) extent of tears.**

	Control			Linear cut			Linear & circumferential				Significance	
	0 cm	1 cm	4 cm	1 cm	1 cm	1 cm	1 cm	1 cm	1 cm	1 cm	Overall	Overall circum. tears
Length of tear	0 cm	1 cm	4 cm	1 cm	1 cm	1 cm	1 cm	1 cm	1 cm	1 cm	Overall	Overall circum. tears
	0%	0%	0%	25%	50%	75%	100%				Control vs linear 1 & 4 cm tears	0 vs 25-100% tears
n	7	20	12	23	19	16	12					
Pressure of water (cm H <sub>2</sub> O) mean±std; median [range]	43.7±18.6 47 [19.5-61]	26.4±23.0 18.1 [0.7-69.7]	23.3±14.4 20.0 [5.5-55]	27.7±21.3 22.0 [4.6-69.3]	33.1±22.1 33.5 [1-67.3]	23.8±24.6 9.3 [1.7-72.0]	24.4±23.9 16.6 [0.9-67.3]				0.18	0.71

\*The Kruskal Wallis test was used to compare perforation pressures with the length of the tear and for pressure required to perforate 1 cm tears with increasing circumference of tear.

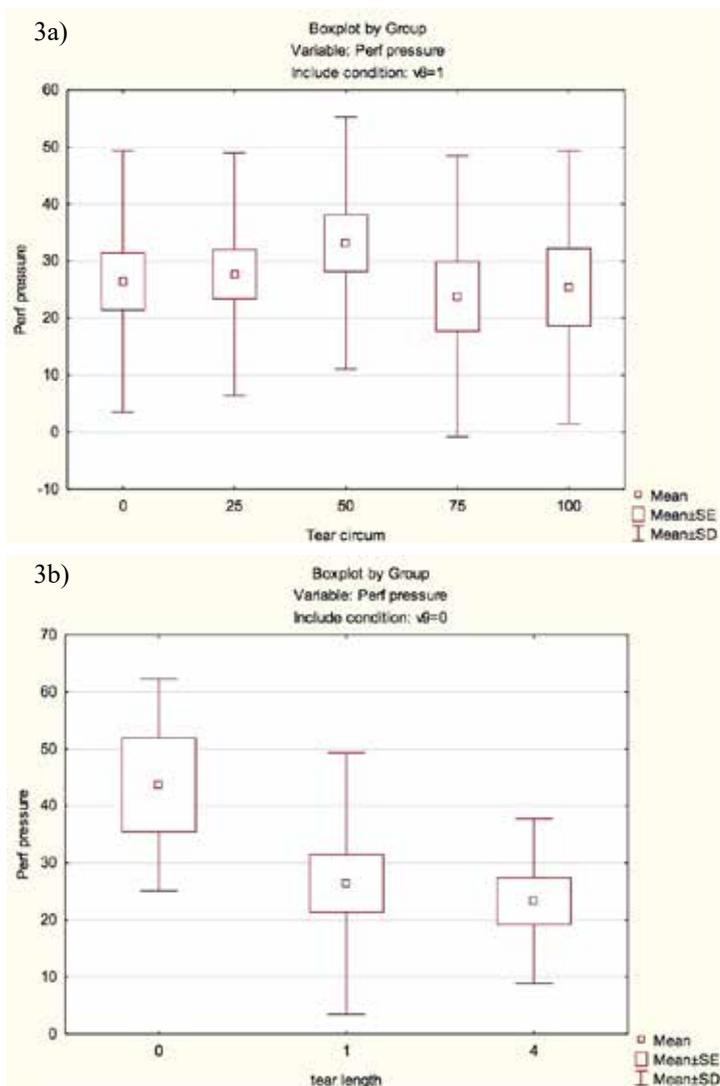


Figure 3. Rupture pressure of bowel in cmH<sub>2</sub>O following the creation of 1cm wide serosal injuries of increasing circumference (a) and following the creation of a 1 cm and 4 cm long longitudinal serosal injury (b).

### Statistical analyses

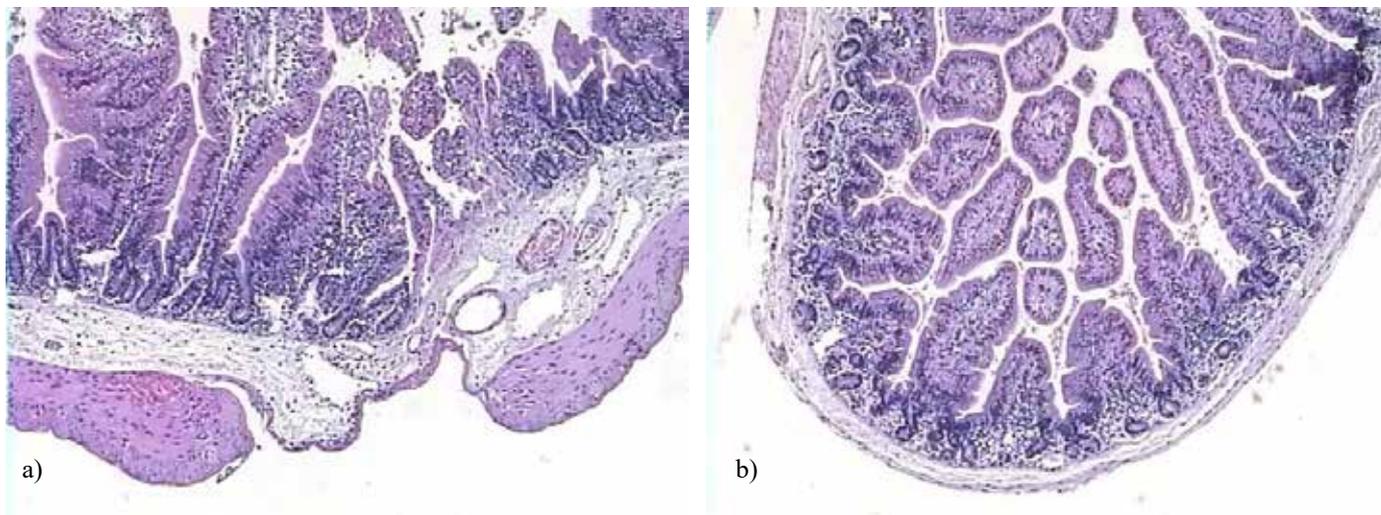
Descriptive statistics as appropriate were used to present study. The Kruskal Wallis test was used to compare perforation pressures with the length or circumference of the various injuries. A  $p < 0.05$  was considered statistically significant where appropriate. The programme ResData 122011 was used for statistical analyses.

### Results

Bowel perforation pressures for normal bowel and the range of circumferential and longitudinal mucosal injuries are shown in Figures 3a and 3b respectively. No difference in rupture pressure between normal bowel and bowel with serosal injuries was noted ( $p = 0.71$ ,  $p = 0.18$  respectively). No perforations were observed at physiological intraluminal pressures of 8–10 cm H<sub>2</sub>O. Perforation occurred at 43.7±18.6 cmH<sub>2</sub>O, 23.3±14.4 cmH<sub>2</sub>O and 24.4±23.9 cmH<sub>2</sub>O for controls, 4 cm long and 100% circumference serosal injuries respectively as shown in Table 1 and Figure 3. In experiment 2, no animal developed a spontaneous bowel perforation during the observation period.

In experiment 1, microscopic examination of the bowel wall using standard H&E staining demonstrated no evidence of ischaemia at the site of perforation.

Figure 4. (a) Standard H&E technique staining of a bowel segment, showing no evidence of ischemia at the site of perforation. All layers of small bowel were viable, (b) Standard H&E technique staining of a bowel segment showing infiltration of inflammatory cells. No signs of ischemia were demonstrated.



All layers of small bowel were viable, and no inflammatory infiltrate was seen as shown in Figure 4a. In experiment 2, the small bowel demonstrated an inflammatory infiltrate at the site of serosal injury; however, no mucosal or submucosal ischaemia was present. The depth of incision in all specimens from both experiments involved the serosal layer only, as shown in Figure 4.

## Discussion

It is recommended that large serosal injuries (involving more than 80% of small bowel circumference) should be managed by a segmental bowel resection;<sup>1</sup> however, there is no reliable data on which length of serosal injuries will result in bowel perforation. Iatrogenic serosal injuries during laparotomy are not uncommon, especially during relook procedures. It may not be feasible to repair each serosal injury and repair may result in small bowel stenoses, especially in paediatric patients. Areas with extensive serosal injuries may require resection and anastomosis which carries a 2–4% risk of postoperative stricturing.<sup>6</sup> Mortality rates as high as 22% have been reported with an anastomotic leak,<sup>7</sup> and the overall morbidity associated with a diverting stoma post-repair or for exteriorizing the anastomotic leak may be as high as 40%.<sup>8</sup>

Anatomy textbooks describe the muscular and submucosal layers as being the strongest layers of the bowel wall. Whether or not serosal injuries result in localized bowel wall ischaemia and ultimately perforate is an assumption and has not clearly been proven.

The data in this article show that serosal injuries perforate at intraluminal pressures beyond physiological pressures of 8–10 cmH<sub>2</sub>O.<sup>3</sup> Hence, the small bowel wall in the presence of large serosal defects, does not appear to be weakened. This with regard to immediate as well delayed perforation.

A limitation of the study is that the rabbits used were healthy animals without risk factors for poor wound healing

commonly present in humans undergoing laparotomy, such as sepsis, organ failure and other immune-compromising states. However, the intra-abdominal milieu in the group of patients that have stable physiology and no immune compromise is probably similar to this rabbit model. Intraoperative assessment in humans can be challenging. Distinguishing true isolated serosal injuries from injuries involving the deeper layers as well can be difficult, especially in inflamed and oedematous bowel. Whether these injuries require surgical repair to prevent perforation requires further experiments. Finally, this is an animal model study and the findings cannot necessarily be translated to humans. The bowel wall of all mammals consists of five layers with the small bowel of an adult-sized rabbit being similar to the small intestine of a full-term human baby in terms of diameter and wall thickness.

In conclusion, small bowel serosal injuries up to 4 cm in length or 100% in circumference do not seem to perforate at physiological pressures of 8–10 cmH<sub>2</sub>O, encountered during normal peristalsis. Furthermore, the tensile strength of the bowel wall and blood supply of the small bowel do not seem to be affected.

## Conflict of interest & funding information

The authors report no conflict of interest or commercial interest in any product mentioned or concept discussed in this article. The project was supported by funding from the Department of Surgery, University of Witwatersrand.

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## Ethical approval

University of Witwatersrand guidelines for the care and use of animals were followed. All procedures performed in this study were in accordance with the ethical standards of the University's Animal Ethics committee and were supervised by the Central Animal Services. Ethics clearance number: 2012/04/05 and 2013/38/2B.

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