

**New experimental surgical techniques for paediatric bladder augmentation
and continent cutaneous diversion**

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THE LIST OF PUBLICATIONS ON WHICH THE THESIS WAS BASED ON

I, **Cervellione RM**, Bianchi A, Fishwick J, Gaskell SL, Dickson AP.

Salvage procedures to achieve continence after failed bladder exstrophy repair.

J Urol 2008;179:304-6. **IF 3.952**

II, **Cervellione RM**, Varga G, Hajnal D, Erces D, Kaszaki J, Harwood R, Rakoczy G, Cserni T.
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III, Cserni T, **Cervellione RM**, Hajnal D, Varga G, Kubiak R, Rakoczy G, Kaszaki J, Boros M, Goyal A, Dickson A. Alternative ileal flap for bladder augmentation if mesentery is short.

J Pediatr Urol 2015;11:64. **IF: 1.170**

IV, **Cervellione RM**, Hajnal D Varga G, Erces D, Kaszaki J, Rakoczy G, Cserni T.

Mucosectomy impairs ileal microcirculation and results in flap contraction after experimental ileocystoplasty.

J Pediatr Urol 2017;13:81.e1-81.e5. **IF: 1.170**

V, **Cervellione RM**, Hajnal D, Varga G, Rakoczy G, Kubiak R, Kaszaki J, Boros M, Harwood R, Dickson AP, Cserni T. New alternative Mitrofanoff channel based on spiral intestinal lengthening and tailoring.

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1. INTRODUCTION

Despite the advanced medical technology severe congenital malformations like bladder exstrophy, cloacal exstrophy and severe neurogenic bladder dysfunction in myelomeningocele patients remain a major challenge in paediatric urology. Salvage procedures to improve urine storage capacity (bladder augmentation) and procedures providing continence, like catheterisable continent stomas (Mitrofanoff channels) are still playing significant role in the treatment of the patients (Cervellione et al. 2008).

At present, the ileocystoplasty is the most accepted and reliable method of bladder augmentation in children with Mitrofanoff stoma. However there are several unresolved concerns regarding the mucus production of the ileal flap within the augmented bladder, the metabolic complications due to the absorption of urinary electrolytes through the intestinal mucosa, the high risk of carcinogenesis and the short mesentery in children with ventriculo-peritoneal (VP) shunt which can make augmentation difficult (Adams et al. 2012, Kropp et al. 2007).

Paediatric urologists are continuously working on to leave the intestinal mucosa out of the augmented bladder. Use of sero-muscular or sero-musculo-submucosal intestinal flaps has recently become more and more popular, but flap contraction still remains major concern (Kropp et al. 2007, Salle et al. 1997). Extensive research is now focusing on how to cover the raw surface of the ileal flaps after mucosectomy with cell cultured urothelium (Turner et al. 2011) believing the urine exposure to the raw surface might be responsible for flap contraction. However the mucosectomy disrupting the intramural vascular circuits may have significant ischemic effect on the microcirculation, which could be responsible for flap contraction alone.

The intestine has traditionally been detubularised along the mesenteric line to perform bladder augmentation. However paramesenteric detubularisation would allow longer reaching flaps, which would be beneficial in patients who have a short mesentery. Ligation of few vasa recta in the mesentery and perhaps the combination of the two procedures would also facilitate tension free augmentation, but there is no data available in the literature regarding the safety of these procedures.

Most of the time the appendix is used for catheterisable channel, but if this is not available or it has been used for other purposes, like a Malone antegrade enema channel, the ileum has to be used. Moreover, the length of the channel that can be created using the Monti procedure may not be long enough, especially for wheel chair bound obese children. Double Monti and Casale procedures result often kinking and obstruction of the channels and often a surgical revision is required. There is a clear demand for long, straight and viable channels (Montiet al. 1997, Casal et al. 1999).

In vivo microscopy using orthogonal polarizing spectral imaging (OPS) is a well-established sensitive technique to monitor tissue microcirculation real time. Since the impact of surgical design and intervention to the tissue can be monitored directly, this technology can open new boundaries in reconstructive surgery (Cerny et al. 2007, Bajory et al. 2012).

2. AIMS

In this program, our aim was to review the importance of the bladder augmentation and catheterisable continent stomas in the clinical practice after failed bladder exstrophy repair (I.), to study the intramural microcirculation of the intestinal flaps used for bladder augmentation using *in vivo* microscopy (II.). We wanted to reveal the undiscovered functional reserves and limitation of microvasculature and the efficacy of intramural vascular anastomoses to design new procedure, which makes bladder augmentation safer and easier when mesentery is short (III.), to better understand the vascular consequences of mucosectomy (IV.) and to create longer and straighter catheterisable channels (V.).

3. MATERIAL AND METHODS

Patients and methods: We retrospectively reviewed the records of 32 patients who underwent our standard protocol of treatment. A total of 21 referred patients and 11 patients treated at our exstrophy unit underwent salvage continence procedures after failed reconstruction. Continence was defined according to the International Children's Continence Society terminology, as continent, intermittently continent and continuously incontinent.

Research animals: The experiment was approved by the Committee of Animal Research at the University Szeged (Permission no: I-74-14/2012 MÁB and V./1637/2013) The study was performed on anaesthetised Vietnamese mini pigs (weight: 25-30 kg).

Orthogonal polarization spectral imaging: The OPS imaging technique (Cytoscan A/R, Cytometrics, PA, USA) was used for visualization of the microcirculation of the mucosa. Microcirculatory evaluation was performed off-line by frame-to-frame analysis of the videotaped images. The capillary red blood cell (RBC) velocity ($\mu\text{m s}^{-1}$) changes were determined and capillary perfusion rate (PR) were calculated from ratio of perfused capillary and total capillary length in the intestinal villi in 3 separate fields by means of a computer-assisted image analysis system (IVM Pictron, Hungary).

Intramural microcirculation of the ileal flap:

Examination of antimesenteric intramural vascular anastomoses: 10 cm-long jejunal loops were isolated in anaesthetised pigs (n=5). *Control Group:* after antimesenteric incision the bowel loop

was opened and the mucosal microcirculation has been recorded with orthogonal polarisation spectral (OPS) imaging technique at the antimesenteric edges of the opened bowel strip. In *Group 1* longitudinal incision was performed on the bowel loop between the mesenteric and antimesenteric border in the middle. After detubularisation the microcirculation has been recorded at the edge of the opened bowel strip beyond the antimesenteric line. In *Group 2* the loops were cut next to the mesenteric line. After detubularisation the microcirculation has been recorded at the edge of the opened bowel strip beyond the antimesenteric line.

Examination of longitudinal intramural vascular anastomoses: Mucosal microcirculation was recorded on a continuous jejunum after antimesenteric incision. Ligation of 2, 4 and 6 neighbouring vessel was performed sequentially and the microcirculation was recorded on the bowel at the midpoint of these vessels. There was some irregularity of vessels in the mesentery of the pigs. Maximum 2 vessels running next to each other were considered as vasa recta (VR). The same study was done on a jejunum segment with free end starting the ligation from the free end of the bowel loop and measuring the microcirculation at the free end.

Effect of mucosectomy on flap microcirculation and flap contraction: Clam ileocystoplasty was performed using 15 cm long ileum segments in 2 groups. First the ileal segment was isolated detubularised along the paramesenteric line. The detubularised bowel strips were placed on wet gauze and the width was measured with linear ruler under no tension and the microcirculation was recorded on the serosal surface. Then mucosectomy was applied. In the seromuscular group the mucosa and the submucosa was peeled off the seromuscular layer in one piece. In the sero-musculo-submucosal group only the mucosa was scraped off with the back of a forceps from the bowel at the level of the mucosa propria. The microcirculation was rerecorded in each group after mucosectomy procedure on the serosal surface. Clam ileocystoplasty was performed in each group with the serosa facing inside (reverse fashion). The denuded surface of the ileal flap facing the abdominal cavity was covered with the omentum in both groups. Malecot catheters (12 F) were left in the urethra for 5-7 days.

New technique for augmentation if mesentery short:

Animals, instrumentation and surgery: In 5 animals adjacent ileal segments were isolated. In control group the ileum was detubularised along the antimesenteric line. In AF group the ileum was detubularised along the paramesenteric line. Subsequent ligation and dissection of 0, 1, 2, 3 and 4 VR was performed in both groups starting from the free end of the segments. The length of the flaps was measured from a point marked with a suture on the base of the mesentery and the difference between the two groups was recorded.

In vivo microscopy was performed to examine the microcirculation of the flaps mucosa. At the end of the measurements the flaps were removed and new 20 cm long ileum was isolated for the clam ileocystoplasty. After four weeks the animals were anesthetized again, and the augmented bladders were examined and removed. Conventional hematoxylin eosin staining and light microscopy examination was performed.

The clam ileocystoplasty: The ileum segment was detubularised along the paramesenteric line on the posterior wall and the continuity of the ileum was then restored with single layer anastomosis. The bladder was incised down to the trigone and the ileum flap was sutured to the bladder using 5/0 Vicryl. 12 F Malecot catheters were placed urethrally.

Histopathological analysis: Full-thickness tissue biopsies taken from the bowel bladder junction on week 4 after surgery and hematoxylin-eosin staining was performed.

New concept to create long and straight continent urinary stomas: Approximately 6-8 cm long piece of ileum adjacent to the ileal bladder flap was mobilised with intact mesenteric blood supply during clam ileocystoplasty. Spiral line was marked on the bowel approximately 15 mm apart with 60° angle to the longitudinal axis of the bowel. When the incision was completed, the mesentery was incised perpendicularly where the spiral incision line met the mesentery. The maximum length segment hanging on a single 1.5 cm wide well vascularized mesentery was detached. The microcirculation was recorded in the middle and at the edges of the bowel strip. The bowel strips were then retubularised in a spiral fashion with interrupted 5/0 Vicryl sutures over a 12 F Malecot catheter and were implanted in the bladder according to the Mitrofanoff principle. The distal end of the channels were brought out through the abdominal wall, sutured to the skin and secured to the fascia and peritoneum. The catheters were secured to the skin with 5/0 Vicryl and were cut at the level of the skin. Another 12F catheter was left in the urethra.

Four weeks later the animals were anaesthetized again and the catheterisable channels were examined and catheterised. Laparotomy was performed, the channels incised and mucosal microcirculation was recorded at the level of the bladder, adjacent to the abdominal wall and in the middle at the level of the mesentery. Histological examination was performed with hematoxylin-eosin staining.

Statistical analysis: The data analysis was performed with a statistical software package (SigmaStat for Windows; Jandel Scientific, Erkrath, Germany). The distribution of our experimental data was analysed by the Kolmogorov-Smirnov normality test. Failure of the normality test indicated nonparametric distribution of the data. Accordingly, we employed nonparametric statistical tests. Differences between groups were analysed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method for pairwise multiple

comparison in Study II. and V. Friedman repeated measures analysis of variance on ranks and Mann-Whitney Test were applied within groups in Study III. Wilcoxon rank sum test was applied for within group analysis. Differences between groups were analysed with Mann-Whitney test in Study IV. In the Figures, median values and 75th and 25th percentiles are given; *p* values < 0.05 were considered significant.

4. RESULTS

Clinical importance and efficacy of the bladder augmentation and continent cutaneous diversion : A total of 32 salvage continence procedures were performed at a mean patient age of 6.3 ± 3.2 years. A total of 29 patients (91%) are continent, 3 (9%) are intermittently incontinent and none is continuously incontinent. One patient is continent after bladder augmentation (BA) using CIC. Two are continent and 1 is intermittently incontinent after BA and bladder neck repair (BNR) using urethral CIC. One is continent using urethral CIC through a cutaneous continent diversion (CCD) into a sigmoid bladder. A total of 19 children are continent after BNC, BA and CCD using CIC. However, 1 patient has required a repeated procedure because of a leaking stoma. A Monti tube was passed through the posterior rectus sheath and skin tunnel was constructed to lengthen the catheterizing channel. Four children are continent and are satisfied with a cutaneous urinary diversion. Two patients are continent and 2 are intermittently incontinent after a Mainz II pouch.

Intramural microcirculation of the ileal flap:

Examination of antimesenteric intramural vascular anastomoses: There was no significant difference in RBC velocity and the perfusion rate at the site of measurements between the Control Group and Group 1-2.

Examination of longitudinal intramural vascular anastomoses:

Continuous loop: No significant changes in the RBC velocity and perfusion rate have been seen after ligation of 2 vasa recta. However the RBC velocity dropped significantly after ligation of 4 vasa recta, but the change in the perfusion rate remained non-significant. After ligation of 6 vasa recta both parameters approached the 0 level.

Loop with the free end: No significant changes in the RBC velocity and perfusion rate have been seen after ligation of 2 vasa recta. The RBC velocity diminished significantly after ligation of 4 vasa recta in an similar manner to the continuous loop. The reduction in perfusion rate was more expressed and significant too. After ligation of 6 vasa recta both parameters approached 0.

Effect of mucosectomy on flap microcirculation and flap contraction: The mean width of the detubularised ileum was 37 ± 1 mm at pre-augmentation in the animals. Significant reductions in RBCV and PR were detected after mucosectomy in both mucosectomy groups. The animals recovered very quickly after the operation. They were not lethargic no loss off appetite was observed and their wound healed well. No complication like peritonitis, ascites was seen. At the autopsy no perforation or necrosis was seen. The average width of the ileum measured after autopsy was 16 ± 2 mm in the sero-muscular group, and 20 ± 2 mm in the sero-musculo-submucosal group. The omentum was found firmly attached to the ileal flaps; however significant ($p < 0.05$) contraction of the flaps had developed in both groups.

New technique for augmentation if mesentery short:

Flap length: Paramesenteric detubularisation of the ileum resulted in mean 20.25 ± 0.5 mm longer flap versus detubularisation along the mesenteric line. The difference in length reached 98% of the mean bowel width (20.5 ± 0.57 mm) measured in the animals. Ligation of each vasa recta further increased the length of both flaps. The length gain was different after each vessel, mean: 10.59 ± 3.18 mm.

Microcirculation: The statistical analysis did not show significant difference in the RBCV and PR between the antimesenterically detubularised ileal flaps and the paramesenterical detubularised flaps. Ligation of vasa recta up to 1 vessel did not affect RBCV and PR compared to the baseline values in both groups. Ligation of 2, 3 and 4 vessels gradually decrease RBCV and PR in both groups significantly, however after ligation of the 4th vessel still marked circulation was seen.

Bladder augmentation: All animals recovered after the clam ileocystoplasty performed with paramesenterically detubularised ileum flap. There was no urine leakage or suture break down found at the autopsy.

Histology: The hematoxylin eosin staining confirmed viable bowel flaps anastomosed to the bladder. The bowel mucosa was not atrophic, no fibrosis seen in the bowel flap.

New concept to create long and straight continent urinary stomas: The mean width of the ileum was 20.5 ± 0.57 mm. We were able to create straight spiral channels over a 12 F Foley catheter with the mean length of 100 ± 26.4 mm. The RBCV dropped 17 % and the PR dropped 8.3 % at the edges of the bowel strip after the spiral incision ($p < 0.05$). However no significant difference was found 4 weeks later.

The Malecoth catheters were lost within 2 weeks. At the level of the epidermis the opening of the channels were found to be narrow, but after some dilatation they became patent and easily

catheterisable. All implanted channels remained viable and straight. The histology showed no necrosis of mucosa and muscle layers either at the skin or bladder level.

5. DISCUSSION

Clinical importance and efficacy of the bladder augmentation and continent cutaneous diversion:

Successful primary anatomical closure of bladder, posterior urethra, abdominal wall and pelvic bones is the first step towards good bladder volume in children born with bladder exstrophy. Pelvic osteotomy allows a tension-free abdominal closure that consistently reduces the chance of breakdown (Meldrum et al. 2003). In this series all of our patients underwent posterior pelvic osteotomy performed by our team.

Gearhart et al reported that patients undergoing 2 or more attempts at bladder closure fail to achieve satisfactory bladder capacity and are not suitable for bladder neck reconstruction (Gearhart et al. 1996). Three of the 21 patients treated elsewhere required a second attempt at bladder closure because of wound breakdown, which compromised the bladder capacity.

Adequate bladder capacity failed to develop in some patients who underwent successful primary bladder closure. We believe that adequate outlet resistance following primary bladder neck closure is fundamental to achieve good bladder capacity. This outcome must be balanced against the need to avoid too tight a closure so as not to compromise the upper urinary tracts.

Bladder neck reconstruction is the next step toward continence for the majority of patients who continue to be wet after primary bladder and urethral reconstruction. Six patients in our series (3 referred) had a failed bladder neck repair and therefore proceeded to salvage continence surgery. The normal expected bladder capacity at age 5 years, the age when we start considering continence surgery, is 180 cc (Neveus et al. 2006.) but similar values develop in few patients with exstrophy. We do not perform bladder neck repair in patients with a capacity of less than 85 cc (half expected capacity), since the literature suggests that bladder neck repair offers more reliable results when performed above this value (Surer et al. 2001.) Bladder neck closure with augmentation and catheterisable stoma may be the solution for these cases.

Four patients who had undergone previous multiple operations underwent cutaneous urinary diversion and are satisfied with that choice. Cutaneous urinary diversion (non continent ileal conduit) should be considered as an alternative to continent cutaneous diversion, particularly if the expected compliance to CIC is likely to be poor. Cutaneous urinary diversion is easily managed, reduces the risks associated with continent stomas and bladder augmentation (urinary

tract infection, stone formation, malignancy), and can be converted to bladder augmentation and continent cutaneous diversion later in life.

Our limited experience with the Mainz II pouch has not been satisfactory, and we do not regularly offer it. Furthermore, we remain concerned at the reported suggestion of a significant incidence of long-term malignancy (Schroder et al. 2006.).

We wait until the patient is at least 5 years old before considering any continence surgery, so he/she can express his/her own view on the matter. The selection between bladder neck repair and the different forms of urinary diversion is mainly based on bladder capacity but the expectation and motivation of the patient and his/her family also has a vital role.

Intramural microcirculation of the ileal flap: Anatomists have extensively studied intestinal circulation in the past, but several controversies have been left unanswered. According to Jonnesco (1912) the vasa recta of the bowel divide into two equal branches, which encircle the gut to anastomose with each other at the antimesenteric border (Jonnesco et al 1919). Eisberg (1924) and Noer (1943) claimed that there are numerous anastomoses between the smaller branches of adjacent vasa recta in the bowel wall. Cokkinis (1930) observed that the intramural vessels ramify in an arborescent manner, but he found no evidence for anastomoses of the contiguous vessels. It has been reported that the subperitoneal ramification stops short a little distance from the antimesenteric border and a clear longitudinal band free from visible vessels is present in all specimens (Eisberg 1943, Cokkinis 1930). Doran studying the upper jejunum in human post mortem specimens found communications only of the arteries of the calibre of the primary branches of the vasa recta. Neither the smaller arteries, nor of those arising from the sides of the larger vessels showed communications. The position of these anastomoses was found irregular and not conforming to any set pattern (Doran et al. 1950).

In our study, we demonstrated undisturbed microcirculation far beyond the antimesenteric line right up to the mesenteric line in a bowel segment, which was detubularised next to the mesentery. In the clinical practice it means that at detubularisation the surgeon does not need follow the antimesenteric line, it can be done anywhere else along the circumference safely without compromising microcirculation. This is in accordance with our experience with the SILT technique we recently developed. In this procedure the intestine is incised along a spiral line. SILT proved viable in animal model and in clinical practice (Cserni et al. 2011).

In clinical practice, paramesenteric detubularisation has only previously been used in a modified Monti procedure to create a catheterisable channel for augmented bladder (Monti et al. 1997). The aim of the paramesenteric detubularization was to leave sufficient length mesentery free end

of the channel to allow easy implantation in the bladder in an antireflux manner to achieve continence. There were no major concerns reported regarding viability of these channels.

Urologists have made several attempts to create a reliable, but “mucus free” bladder augmentation, but this still remains unsolved. In an experimental animal study “mucus free” bladder augmentation has been performed with paramesenterically detubularised reverse sero-muscular bowel flaps. All the flaps fibrotized and contracted within a few weeks (Cheng E et. al 1994). It was not clear whether the paramesenteric detubularisation or the mucosectomy was responsible and the technique was abandoned. It is now clear the paramesenteric detubularisation cannot be claimed to be the reason for this. Our team was able to perform successful bladder augmentation with paramesenterically detubularised flaps in experimental settings without mucosectomy. We are now focusing on less traumatic mucosectomy and are getting closer to achieve “mucus free” augmentation.

Detubularisation of the ileum next to the mesentery rather than at the antimesenteric line may allow us to create longer reaching vascularised ileal flaps for bladder augmentation during clam ileocystoplasty, which is particularly useful if the mesentery is short. This could be also useful for vaginal replacement in cloacal anomalies when the bowel segment needs to reach to the perineum.

To examine the efficacy of the longitudinal anastomoses the antimesenteric anastomoses were cut. The bowel wall received perfusion only through the neighbouring longitudinal anastomoses at the site of the measurements after the corresponding vasa recta had been cut. On the continuous loop the blood supply came from both sides, while on the loop with the free end only from one side.

The fact that ligation of a 2 vasa recta did not affect the microcirculation is a clear evidence of the presence of functioning longitudinal intramural anastomoses. The efficacy of these anastomoses was limited up to ligation of 4 vessels. We expected the loop with the free end to be more sensitive to vasa recta ligation having anastomoses only from one side, but we did not find a marked difference between the two loops. The reduction of the RBC velocity was very similar, only the perfusion rate dropped more rapidly in the loop with the free end. These findings are not really surprising and justify the present surgical practice that only very short bowel segment detached from mesentery can be used safely

Effect of mucosectomy on flap microcirculation and flap contraction: With the spread of minimally invasive endoscopic submucosal dissection (ESD) technique for removal of gastrointestinal mucosal malignancies, there is more indirect evidence that oesophageal, gastric and colorectal stricture occurs after surgical manipulation of the submucosa, especially after

ESD of large lateral spreading tumors occupying 75% of the lumen (Shoji et al. 2013, Kwon et al. 2014). Further evidence of contraction after mucosectomy, is the cuff stenosis which occurs after the Soave endorectal pull through procedure for Hirschsprung disease, where during the procedure submucosal dissection is carried out (Dasgupta R et al. 2005). Indeed it is general practice to incise the residual seromuscular cuff, such is the regularity with which cuff contraction occurs.

In this study we used *in vivo* videomicroscopy to monitor the microcirculation of the seromuscular ileal flaps in real time after mucosectomy. The two parameters (the velocity of the circulating red blood cells and the ratio of the open and closed capillaries) measured are widely accepted parameters to represent the patency of microcirculation (Czerny et al. 2007). These findings clearly indicate that mucosectomy with or without removal of the submucosa has major impact on the microcirculation of the residual flap. The incomplete circulatory cessation explains why acute necrosis and perforation was not present and why histology with light microscopy did not reveal thrombotic vessels in previous studies.

It has been observed that the branches of the vasa recta encircle the intestine and penetrate the muscle layers and form an arterial plexus within the submucosa. The vessels supplying the mucosal and muscular layers of the gastrointestinal tract originate from this plexus (Kvietys et al. 2010). We propose that mucosectomy may disrupt fine vascular circuits within the intestine and the blood supply of the mucosectomised bowel may rely only on residual vessels originating directly from vasa recta. However temporary ischemia-reperfusion caused by mucosectomy cannot be ruled out in the background.

It could be postulated that exposure of the serosa to urine could be responsible for contraction of the flaps and we should have used a control group with intact reversed flaps with no mucosectomy to assess this question. In a dog experiment, however, Cheng et al clearly showed that the serosal surface of the reversed ileal flaps become epithelised by urothelium and intact (full thickness) reversed flaps did not contract (Cheng et al. 1994).

The application of intact (full thickness) reversed flaps is not ideal either, because the mucus produced by the intact flaps inside the peritoneum may result in intra abdominal mucocele formation. Cheng et al. reported moderate amounts of mucus pooled intraabdominally one month after surgery in a dog. We also found a large mucocele perforating to the augmented bladder in one pig with full thickness (no mucosectomy) reverse ileal flap augmentation. The ileum flap however remained wide, i.e. no contraction seen. To comply with our ethical approval (to keep the number of animals sacrificed as low as possible) we did not use more animals for full thickness reverse flap augmentation. Splints may have prevented the flap contraction, but the pig

bladders in our study were not drained for a long time, the animals lost their urethral catheter within 7 postoperative days.

The omentum is known to be highly vascularized with microvascular endothelial cells and is composed mainly of adipocytes that produce an enormously high level of vascular endothelial growth factor (Xu et al. 2014). Omentopexy has been used to facilitate revascularisation of trachea grafts, myocardium and even brain (Kainuma et al. 2015), however in our experiment it failed to recover the mucossectomised flaps. Revascularisation from the omentum may require a longer period of time to occur and should precede the ischaemic event, which is obviously not possible in this situation.

New technique for augmentation if mesentery short: Tension-free bowel anastomosis and well-perfused tissue are essential when bowel segments are used in reconstructive urological procedures. Short mesentery due to previous surgeries, peritonitis, peritoneal dialysis and VP shunt may complicate these procedures (Adams MC 2012). Levin used the “stepladder incision” technique and gained up to 30 mm of additional length for urological reconstruction without complication (Levin LA et al. 1992). This is 80% of the diameter of an adult human ileum (37.5 mm) according to Gray’s anatomy textbook. The paramesenteric detubularisation of the ileum resulted in 20.25 ± 0.5 mm longer alternative flaps in the pigs, this reached 98 % of the diameter of the bowel (20.5 ± 0.57 mm). These results suggest that paramesenteric detubularisation is more effective than Levin’s procedure. Further benefit could be achieved if both techniques were combined. Intravital microscopy showed no significant difference in the velocity of the circulating erythrocytes and the perfusion rate at the edge of the alternative ileal flaps. All 5 operated animals survived without suture break down or urine leakage and the histology did not reveal necrosis at the anastomosis line. These findings demonstrate that paramesenteric detubularisation is safe and does not compromise the microcirculation of the ileum and wound healing.

According to the observation of Eisberg HB et al. 1943, we expected that ligation of some VR did not alter microcirculation. Ligation of 1 VR did not alter the RBCV and PR significantly either in the control or in the AF groups. This may suggest that paramesenteric detubularisation and ligation of 1 VR could be combined to gain more length. In clinical practice however, due to the possible anatomical variations, it could be difficult to assess the limitation of the VR ligation unless precise intraoperative intravital microscopy is performed. Although there are no exact data available in the literature regarding the values of RBCV and PR required for uneventful wound healing, in this study we hypothesized that any compromise in the microcirculation may result in complications.

New concept to create long and straight continent urinary stomas: The concept of Spiral Intestinal Lengthening and Tailoring (SILT) i.e. refashioning normal calibre bowel tube to a narrower shape in order to create an alternative Mitrofanoff channel was tested in this study.

The channel length achieved by SILT in this experiment (100 ± 26.4 mm) is outstanding, it exceeds the calculated length of the Monti technique (bowel width x 2) and the double Monti and the Casale procedure (bowel width x 4).

Theoretically, catheterisable channels could be designed by determining the length of the channel required and calculating backward the length of segment needed to harvest based on percent increase in length and tailoring. In our model we experienced 55 % lengthening and 80 % tailoring, this is in accordance with the linear regression between the lengthening and tailoring in SILT we reported earlier (Cserni et al 2011). However in this model it became clear that 2 full (360 degree) spiral cuts, one proximal and one distal to the mesenteric attachment selected, provides a longer channel compared to present techniques without bulky mesentery at the end of the channels for easy implantation. The length of the harvested bowel segment is rather determined by the width of the mesentery selected. This measures 15-20 mm at the Monti or Casale procedures and it is usually enough to incorporate a strong branch of the mesenteric vessel. The mesentery in our model was designed to be 15 mm wide, however a wider mesentery strip could have been easily used. This would have resulted in stronger mesentery and would have further increased the channel length, however beyond a certain extent may have complicated spiral reconstruction. It is more difficult to reconstruct a wide bowel strip to a narrow (12 F) spiral channel. The minimum length of the harvested segment should be 3 times the width of mesentery we select. In our model this was 45 mm. In the study however 60-80 mm long segments were harvested to facilitate tissue handling and half of them was discarded. We believe losing of 40-50 mm bowel length is not significant.

Extrapolating our results to a human ileum, which is approximately 55 mm wide ($\pi \times \text{diameter} / 2$) this creates a spiral channel longer than 220 mm. We believe a channel longer than this is hardly ever required.

A transient alteration in the microcirculation of the bowel immediately after the procedure was experienced. Interestingly, after using SILT in a model of short bowel syndrome, changes in the microcirculation after spiral cut were not recorded (Cserni et al. 2011). This could be explained with the fact that for the alternative Mitrofanoff the bowel was disconnected next to the vasa recta, while in the short bowel model the bowel remained continuous. Furthermore in the short

bowel model dilated (40-50 mm wide) bowel loop was refashioned and the bowel strip after the spiral incision was much wider (30-40 mm) compared to the present experiment where the spiral incision resulted in 15 mm wide bowel strip. The tissue handling could have simply had an effect on the microcirculation, however this was performed with care and the bowel was rinsed regularly with warm saline. Four weeks later normal microcirculatory patterns were found. The channels were patent and histology did not show necrosis or atrophy.

It was possible to reconstruct the bowel strip along the long edge, like in the Monti tube. In this case the strip was kept less than 1 cm wide to get an ideally slim channel, however spiral reconstruction allows cutting a wider (1.5-2cm) strip with more sufficient blood supply.

In humans, catheters are usually left for a few weeks in Mitrofanoff channels and the patients self catheterise (dilate) them on regular basis afterwards to prevent stomal stricture at the skin level. The animals do not tolerate catheters sticking out of their body (we lost our catheters within days) and intermittent catheterisation of the channels is not possible without general anaesthesia. In this study we did not expose the animals to this repeated stress. This is clearly a limitation of our model. This and the fact that pig skin is more robust than human may contributed to the stomal stenosis despite the use of V-shaped stomas in this study. However, we believe viability is the key to the reproducibility of easy catheterisation. The microcirculation data and the histology findings did not suggest chronic ischaemia at skin level.

The Casale channels were reported requiring subfascial revision in 15.2 % versus 8.3 % of the Monti channels (Leslie et al. 2007). The nearly double complication rate may be related to the significant angulation in the middle of the Casale channel, which has to be straightened surgically during its creation. The possible tissue memory at this point, just like the anastomosis in double Monti channels may result in difficult catheter passage, stricture or perforation. The spiral channels were found to be straight. The suture line within the tube runs in spiral fashion, in theory it must guide the catheter tip easily, as the barrel of a rifle guides a bullet. In conclusion, SILT technique is suitable on normal calibre bowel to create an alternative Mitrofanoff catheterisable stoma which is longer and straighter than can be achieved with present techniques. This technique may have some potential for ureteral replacement as well.

6. SUMMARY OF THE NEW RESULTS AND CONCLUSIONS

1, Bladder exstrophy remains a major challenge that is best managed at dedicated centers. Failure of primary bladder closure, inadequate penile reconstruction in boys and unsuccessful bladder neck repair represent the main obstacles to continence and spontaneous voiding. Even at the best centers results may be less than satisfactory and salvage continence surgery remains relevant. Bladder neck closure, bladder augmentation and continent cutaneous diversion ensure that the patient is dry. For those patients with a low leak pressure who tolerate urethral catheterization augmentation with bladder neck repair is a viable alternative.

2. Using state the orthogonal polarizing spectral imaging - state of the art intraoperative real time in-vivo microscopy technique - we first provided direct evidence for safe and reliable antimesenteric and significant, but limited longitudinal intestinal intramural anastomoses in experimental settings.

3. Based on these observations, we modified conventional clam ileocystoplasty for cases when a short mesentery may compromise the success or safety of the procedure. With the paramesenteric detubularisation of the ileum we could achieve significantly longer reaching ileal flaps. This technique does not disturb the microcirculation of the ileal flap and it has been proven to be safe in our animal model. We demonstrated significant longitudinal intramural anastomoses in these flaps as well, but their efficacy was limited and seems not reliable for clinical application.

4. We demonstrated severely compromised microcirculation of the ileal flaps after experimental mucosectomy. The microcirculatory damage may be primarily responsible for flap contraction after bladder augmentation with mucosectomised intestinal flaps. This suggests that future research should focus on the preservation /restoration of the microcirculation during and after mucosectomy rather than reproducing urothelium coverage.

5. In an experimental model, we successfully applied the SILT technique on normal calibre bowel to create an alternative catheterisable cutaneous urinary diversion stoma, which is longer and straighter than can be achieved with the double Monti and the Casale techniques. This technique may have some potential for ureteral replacement as well.

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