

Less-invasive hysterocystoplasty technique: experimental study in goats

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A novel less-invasive hysterocystoplasty technique was evaluated experimentally in goats, via closure of a large defect of the dorsal aspect of the bladder by the uterus. Follow up for six months included radiographic and ultrasonographic examinations, evaluation of kidney function and electrolyte changes, and histological examinations. Microscopically the perimetrium was completely covered with healthy urothelium under which disorganized smooth muscle fibers appeared admixed with slight fibrosis, without demonstrable evidences of shrinkage, infection or malignant transformation. Generally, the technique had many advantages as it required less-invasive procedures, avoided intestinal resection, achieved high survival rate without life threatening complications, caused no significant changes in kidney function or electrolyte levels, and associated with proper healing, acceptable degree of fibrosis, and absence of shrinkage or metaplasia. The technique can be considered as an alternative cystoplasty technique.

Key words: Bladder, Experimental study, Goat, Hysterocystoplasty, Less-invasive technique, Uterus

Although the use of gastrointestinal tissue for reconstruction of the lower urinary tract by invasive techniques is not a new idea (Tizzoni *et al.*, 1888), researches for remodeling of these techniques, to overcome their complications, have never been stopped (Kropp *et al.*, 1996; Matapurkar *et al.*, 2010; Reddy *et al.*, 2000). The main aim of remodeling of these techniques is establishing a capacious, non-refluxing, low-pressure reservoir for urine storage, with achieving minimal fibrosis and shrinkage of the augmenting graft (Lutz *et al.*, 1995; Seif *et al.*, 2007).

Bladder augmentation by gastrointestinal tissues had multiple complications, which included altered electrolyte levels and acid-base balance (Leong *et al.*, 1972; Piser *et al.*, 1987; Balachandra *et al.*, 2007), stone formation, calcium-phosphate metabolism modifications with potential bone growth retardation, gastrointestinal motility disorders, and perforation or malignant transformation of the graft (Nurse *et al.*, 1989; Bauer *et al.*, 1992; Palmer *et al.*, 1993).

Multiple modified cystoplasty techniques using

reversed or non-reversed demucosalized bowel, or non-reversed demucosalized bowel lined with urothelial grafts (Lutz *et al.*, 1995; Shoemaker, 1995) have been described. Although the modified invasive techniques resolved some complications of conventional invasive techniques, variable and unacceptable results were obtained with non-reversed demucosalized bowel patches technique (Shoemaker *et al.*, 1957; Martin, 1959; Salle *et al.*, 1990; Shoemaker, 1995; Barry *et al.*, 1998).

The other less-invasive techniques aimed at elimination of both the need for bowel anastomosis and the direct contact between the urine and intestinal mucosa, via the use of reversed seromuscular layer of intact bowel segment through its anti-mesenteric surface (Shakeri *et al.*, 2009; Aminsharifi *et al.*, 2011). In spite of the observed urothelial and smooth muscle regeneration with these techniques, the overall results were disappointing because of the marked shrinkage of these tissues that contributed to the irritating effect of urine in contact with non-mucosal tissue (Barry *et al.*, 1998). The remarkable regeneration capacity of the bladder (Barry *et al.*, 1998) may explain the success of some of these techniques in improvement of bladder urodynamic variables (Aminsharifi *et al.*, 2011).

The disappointing results of cystoplasty techniques using gastrointestinal tissue excited our interest in enlarging the bladder by the serosal surface of the uterus. The main objectives of the study was to investigate alteration of electrolytes and acid-base balance, evaluation of urothelial regeneration on the serosal surface of the uterus, evaluation of extent of augmenting-patch fibrosis, and finally investigation of possibility of metaplastic changes in this patch.

Materials and Methods

Three healthy native-breed goats were subjected to less-invasive hysterocystoplasty. They were

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prepared for aseptic laparotomy and anaesthetized by diazepam (0.5 mg/kg, i.v.), xylazine HCl (0.1 mg/kg, i.m.), epidural analgesia and local linear analgesia at the site of laparotomy. A midline laparotomy was performed to expose the uterus and the bladder. About 40% of the bladder was excised through its dorsal aspect to create a large defect of the bladder. First the far side of the bladder defect, towards the neck of the bladder, was sutured to the dorsal wall of the uterine body (Fig. 1) and that was followed by the near side for complete closure of the bladder defect (Fig. 2). The abdominal wall was sutured in layers in standard manner. Aftercare of the animal included long acting inj. oxytetracycline every three days by two successive doses, and frequent aseptic catheterization of the bladder for one week.

Blood samples were collected for evaluation of serum total protein, albumin, urea, creatinine, calcium, phosphorus, sodium and potassium levels. These samples were collected before surgery, and every month till the 6th month. Ultrasonographic and radiographic evaluations of the bladder were performed every month post-surgery till the animals were euthanized, six months post-surgery. The transplanted uterine horn, the bladder, and the kidneys were collected and fixed in 10% neutral formalin and processed for routine histologic examination. 5-7 μ m sections were stained with Haematoxylin and Eosin and Masson's Trichrome stains (Bancroft *et al.*, 1992). Biochemical parameters of blood samples were analyzed statistically by one way analysis of variance (ANOVA).

Results and Discussion

From surgical point of view, hysterocystoplasty was a simple, rapid and less-invasive technique, associated with minimal bleeding and avoided the need for intestinal resection and anastomosis with subsequent disturbance of gastrointestinal motility, alteration of electrolytes, and acid-base imbalance (McDougal, 1992; Lutz *et al.*, 1995). All the operated goats were in good health and completed the period

of the experiment (6 months) in contrast to the lower survival rates obtained with less-invasive techniques that used intestinal tissues for bladder augmentation (Aminsharifi *et al.*, 2011).

The serum total protein, albumin, urea, creatinine, calcium, phosphorus, sodium and potassium levels (Table 1) did not reveal any significant changes in their levels, indicating neither alteration of kidney function as a result of ascending infection and inflammation nor resorption of urine through urothelial cracks or the perimetrium. This might be due to absence of contact between the urine and mucosal surface of augmenting patch (Shoemaker 1955; Shoemaker *et al.*, 1957; Lutz *et al.*, 1995; Shakeri *et al.*, 2009; Aminsharifi *et al.*, 2011).

Examination of the bladder, by ultrasonography and radiography, revealed absence of perforation, stone formation, or signs of cystitis, and showed gradual increase of the capacity of the bladder over time.

Gross post-mortem examination showed hypertrophy of the bladder with no pathological alteration in the uterus, bladder, or the kidneys (Fig. 3&4). The inner surface of the bladder and mucosal surface of the uterine graft appeared smooth with no clear signs of cystitis. Suture line showed complete fusion of the graft to the bladder, and no over growths/masses could be detected in the uterine or bladder tissues.

Regeneration and repair constitute two broad categories of healing process that are distinguished by structural and functional characteristics. Regeneration restores tissue organization, architecture, and functions, while repair means that restoration is incomplete or disordered and associated with excessive collagen deposition and in extreme cases, scar tissue formation (Clark *et al.*, 1998; Stocum, 2000).

Microscopically, the perimetrium was covered with multiple layers of transitional epithelium (Fig. 5 & 6). Granulation tissue could be seen in most

Table 1: Mean \pm SD of serum biochemical parameters of operated goats over six months.

Date of sampling (Mean \pm SD)	Before surgery	2 nd Month	4 th Month	6 th Month
Serum total protein g/dL	6.50 \pm 0.2	6.50 \pm 0.2	6.43 \pm 0.25	6.50 \pm 0.17
Serum albumin g/dL	2.5 \pm 0.20	2.43 \pm 0.15	2.57 \pm 0.06	2.47 \pm 0.12
Serum urea mg/dL	27.33 \pm 2.89	25 \pm 4.58	25.67 \pm 0.58	26.33 \pm 2.08
Serum creatinine mg/dL	0.81 \pm 0.09	0.76 \pm 0.03	0.74 \pm 0.02	0.78 \pm 0.04
Serum calcium mg/dL	9.20 \pm 0.70	9.23 \pm 0.29	9.27 \pm 0.55	9.40 \pm 0.52
Serum phosphorus mg/dL	4.63 \pm 0.21	4.50 \pm 0.27	4.60 \pm 0.10	4.63 \pm 0.15
Serum sodium mmol/L	145 \pm 1.0	145 \pm 3.0	144 \pm 1.73	144 \pm 1.0
Serum potassium mmol/L	4.07 \pm 0.15	4.07 \pm 0.15	4.23 \pm 0.23	4.17 \pm 0.06

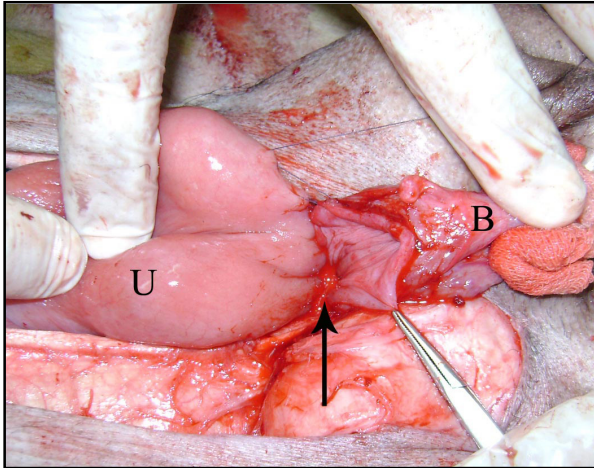


Fig. 1: Suturing of the far side of the bladder defect to the uterus. U: Uterus, B: Serosal surface of the bladder, and the black arrow denotes suture line.

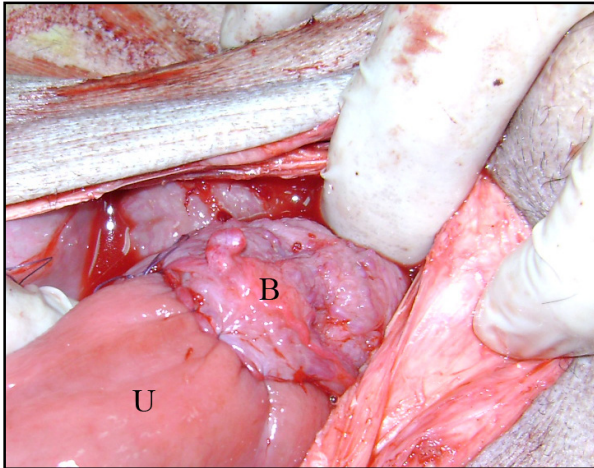


Fig. 2: Suturing of the near side of the bladder defect to the uterus. U: Uterus, B: Serosal surface of the bladder.

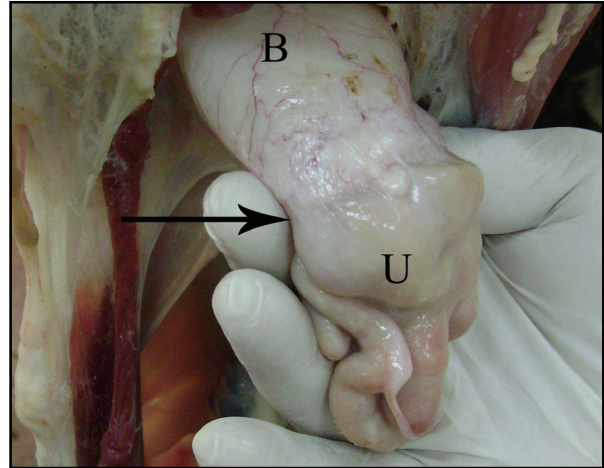


Fig. 3: Gross appearance of the outer surface of the bladder showing the regenerated bladder (B) and the uterus (U), the black arrow denotes suture line.

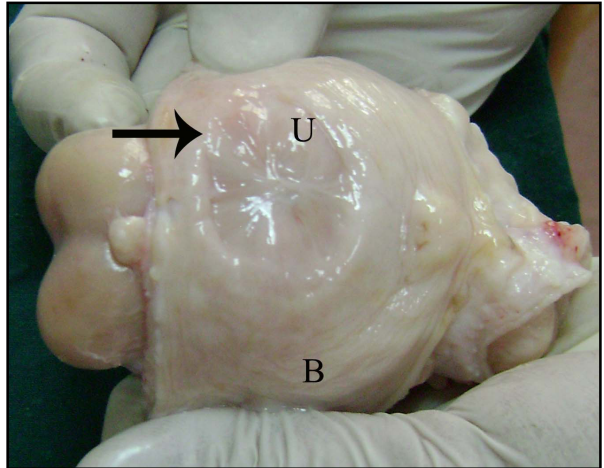


Fig. 4: Gross appearance of the inner surface of the bladder showing the uterine graft (U), the mucosal surface of the bladder (B), and the black arrow denotes suture line.

cases in the form of few number of newly-formed randomly distributed blood vessels, in the sub-epithelial area, admixed with few number of fibrocytes especially within the line of anastomosis (Fig. 7 & 8). The smooth muscle fibers were disorganized with no clear inner or outer layer, but there was no demonstrable evidence of shrinkage or infection. The uterine tissue (perimetrium, myometrium and endometrium) was fully preserved and organized but with urothelium coverage on the peri-metrium, at the same time the native bladder tissue had normal architecture. Formation of considerable amount of granulation tissue and fibrosis result in poor compliance and decreased functional bladder volume (McDougal, 1992).

Metaplastic changes and growth of neoplasms were the most observed serious complications after cystoplasty by intestinal tissues (Hasegawa *et al.*, 1989). Absence of metaplastic changes in this study might be due to complete and proper urothelial covering of the perimetrium, at the same time it emphasises the high resistance of this tissue to the irritating effect of urine, and the high need for further long term studies to prove this theory.

The success of this technique can be explained in the light of the mild fibrosis of augmenting patch, the remarkable regeneration capacity of the bladder (Barry *et al.*, 1998), and absence of severe shrinkage of augmenting patch like that reported by some literatures (Barry *et al.*, 1998). Accordingly,

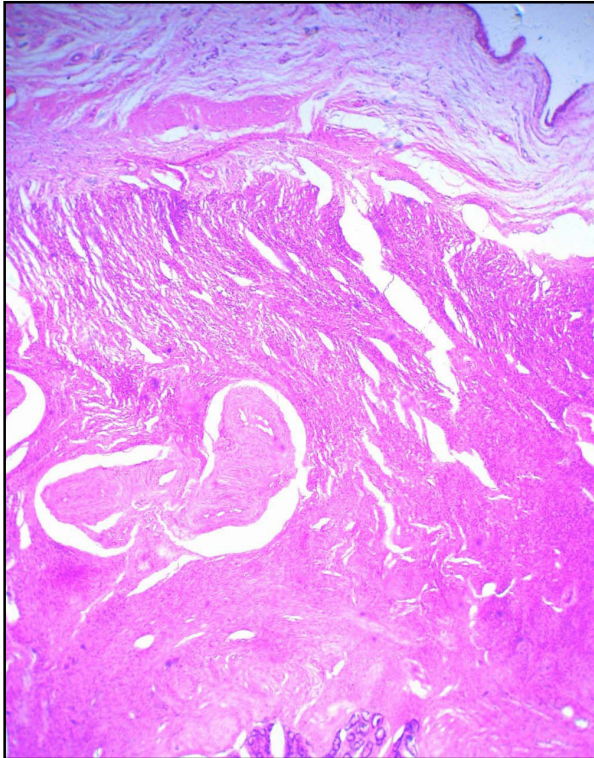


Fig. 5: Complete covering of the perimetrium with urothelium (H&E-X100).

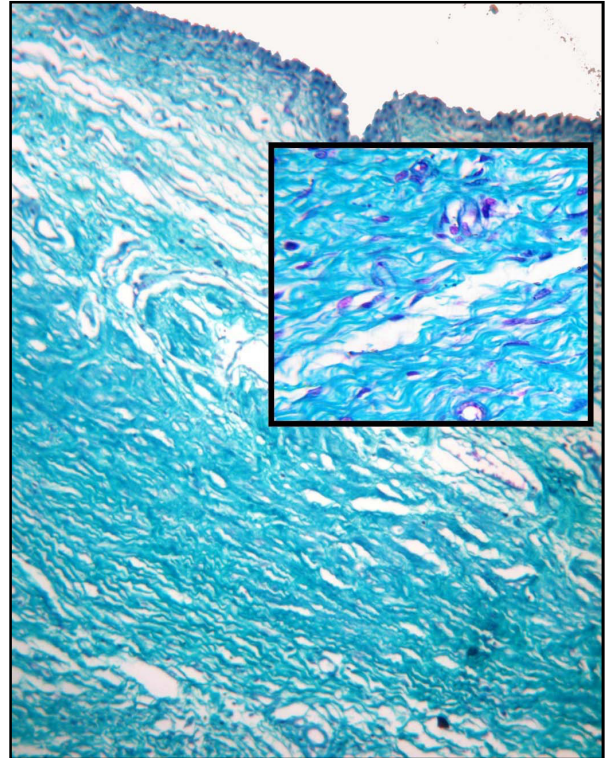


Fig. 7: The submucosa shows newly-formed granulation tissue with randomly distributed blood vessels (Trichrome Stain- X100).

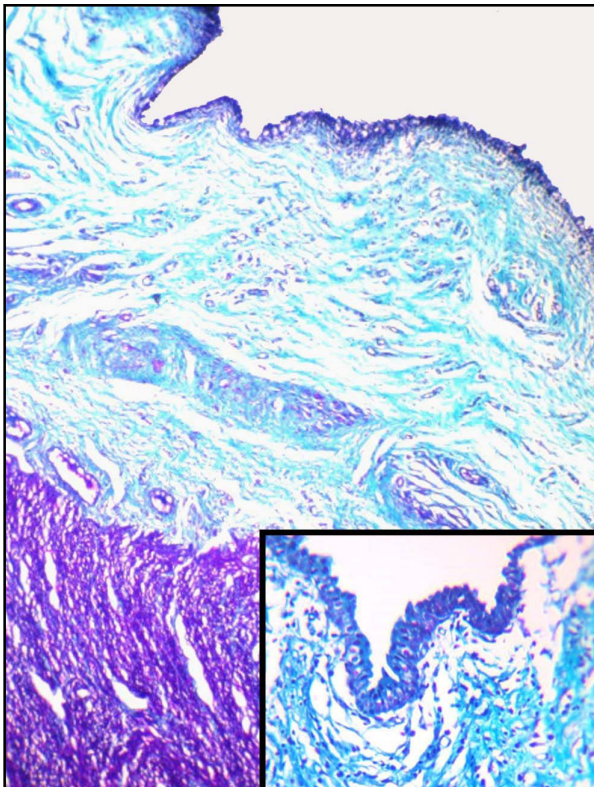


Fig. 6: Complete covering of the perimetrium with urothelium (Masson trichrome stain-X100).

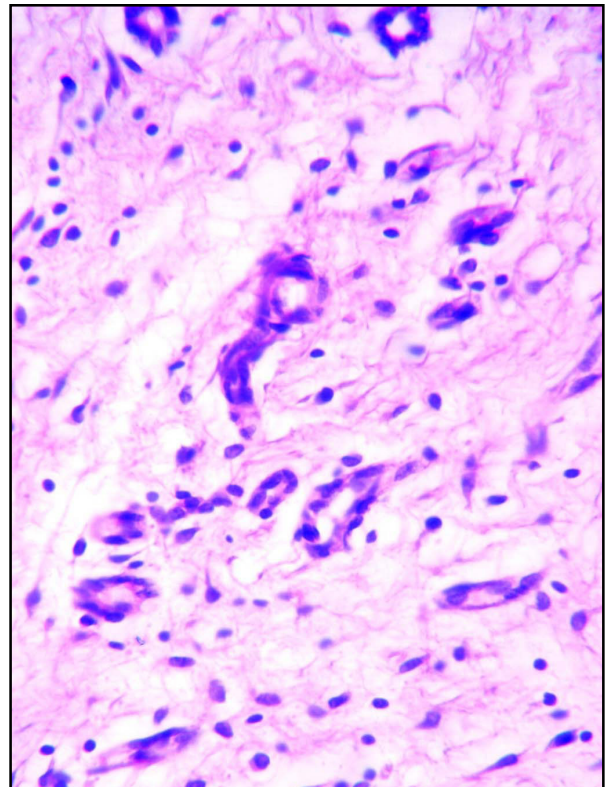


Fig. 8: Well developed fibrous tissue with randomly distributed blood vessels (Trichrome stain-X200).

there was no disturbance of function of the bladder or chronic cystitis in all operated goats.

Less-invasive hysterocystoplasty is an acceptable alternative technique for increasing bladder capacity. The technique required simple procedures and can avoid both the need for intestinal resection and anastomosis, and the direct contact of urine with the mucosa. The degree of fibrosis is acceptable and associated with no clinical disturbance of the bladder function or capacity. Neoplastic changes were not detected six months post-surgery and further long term studies are advised.

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