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ANALYSIS OF BLADDER HISTOLOGY AND URODYNAMICS IN FEMALE RATS SUBMITTED TO BLADDER RECONSTRUCTION WITH RECTUS ABDOMINIS MUSCLE

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ABSTRACT

Introduction: Bladder histology and measures of maximal bladder pressure and maximal bladder volume during urinary loss in female rats submitted to bladder reconstruction with a myoperitoneal flap of rectus abdominis muscle were studied.

Materials and Methods: Fifty female adult Wistar rats were studied, separated in 5 groups: Group 1 (immediate): submitted to laparotomy, cystostomy, and cystometry; Group 2 (sham): submitted to laparotomy, anterior bladder wall incision, immediate suture and cystostomy; Group 3 (cystectomy): laparotomy, partial cystectomy, cystostomy; Group 4 (cystoplasty): midline laparotomy, partial cystectomy, augmentation with rectus abdominis muscle flap peritonized, cystostomy, total cystectomy, the rats were sacrificed and an histological study of the augmented bladder performed; Group 5 (cystostomy): midline laparotomy and cystostomy. Cystometry was always performed in post-operative day 7, excepting for Group 1. Assessment was comparative maximal bladder pressure and maximal bladder volume during urinary loss among the different groups.

Results: In cystoplasty group, mean maximal bladder pressure during urinary loss was lower than in immediate, sham, and cystostomy groups. It was also observed that maximal bladder volume during urinary loss presented mean and median values very close in each group, and cystectomy group showed much lower values. The group submitted to cystoplasty presented mean maximal bladder volume during urinary loss higher than all groups analyzed. Histological analysis of myoperitoneal flap augmented bladders showed partial and/or total epithelization in the muscular flap interspersed region with transitional cells, squamous metaplasia region and chronic inflammatory process.

Conclusion: The use of peritonized rectus abdominis muscle flap to perform bladder augmentation was technically viable in the animals, showing urothelial epithelization in the muscular region of the flap, and a satisfactory gain of capacity and maintenance of low bladder pressures.

Key words: urinary bladder; reconstructive surgical procedures; rats; urodynamics; histology
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INTRODUCTION

Patients with severe urinary bladder deformities, congenital or acquired, may present a functional bladder capacity reduction, as well as alterations in bladder complacence. In the pediatric population, neurogenic bladder due to meningomyelocele and posterior urethral valve bladder are the most common examples of this condition. It is known that whenever intravesical pressure during urinary loss is superior to 40cm H_2O, there is risk of upper urinary tract impairment and, therefore, initial clinical measures must be instituted (1). In the occurrence of absence of response to clinical therapy, the requirement for a reconstructive surgery should be considered, aiming at an organ augmentation, favoring thus the urinary volume storage in a low intravesical pressure.

Countless bladder augmentation techniques using detubularized digestive tract segments were described during the last decade, and are increasingly used as tissue source. However, the existent surgical options may cause several troubles assessed during this patients' late follow-up (2-4).

Theoretically, the best way to treat these cases would be to recommend a bladder reconstruction using an easily available tissue, good blood supply conditions, and that would allow a sufficient bladder capacity gain with adequate complacence. Büyükünal et al. (5) initiated an experimental investigation about bladder reconstruction, using a segment of abdominis rectus muscle of the rat aiming to assess its technical feasibility and perform its histological study. Despite being a pioneer study and relate more than one possible alternative for bladder augmentations, this work has motivated new research that could demonstrate the essential features for the success of a new bladder substitution technique.

Recently, using an experimental model in rabbits, the efficiency of bladder reconstruction technique with rectus abdominis muscle use was assessed through radiological study in 15 animals, and the results suggested its good viability (6).

Thusly, we begin to develop in our facility a work for standardization of comparative analysis of surgical procedures in rat bladder, through the creation of an experimental model defining the features of urodynamics study performance, equipments, catheters, and normal bladder histological pattern in rats (7).

Considering this research line in bladder reconstruction, we started to progress in our experimental investigation in rats, comparatively analyzing urodynamic parameters and histological studies in animals submitted to bladder reconstruction with rectus abdominis muscle, which is the aim of this paper.

MATERIALS AND METHODS

Fifty female adult rats, aged approximately 3 months, weight between 180 and 240 grams (mean=220 grams) were used. The experiment protocol was approved by the Ethics Committee.

In the animal room, the rats were confined in plastic cages with metal latticed cover, maintained in a room temperature of approximately 22°C (71°F), and artificial light with fluorescent lamps. A photoperiod light of 12 hours, intercalate by 12 hours dark was maintained.

The rats received Labina-Purina (São Paulo, Brazil) rations and water ad libitum, and were maintained in the animal room.

Anesthetics used to all animals were intraperitoneal, using 50mg/kg thionembutal. The animals were operated with the aid of 2.5X magnification lenses and were distributed in 5 experimental groups, with 10 female rats each: a)- Group 1 (immediate): submitted to midline laparotomy, cystostomy and 2 catheters (3F) insertion in the bladder dome. The catheters were sutured to the bladder for fixation and exteriorized in dorsal region near the animal head through a subcutaneous route. In this group, right after cystometric study the animals were sacrificed; b)- Group 2 (sham): submitted to midline laparotomy, anterior bladder wall incision, immediate suture, 2 catheters (3F) insertion in the bladder dome; c)- Group 3 (cystectomy): midline laparotomy, half-bladder exeresis and 2 catheters insertion after bladder immediate suture; d)- Group 4 (cystoplasty): midline laparotomy, half-bladder exeresis, and then augmentation with myoperitoneal flap, using peritonized rectus abdominis muscle with approximately 1.5cm length and 1.0cm width, with its blood supply by the inferior epigastric artery (Figures-1 and 2), and also 2 catheters were inserted and fixed in neobladder right superior region; e)- Group 5 (cystostomy): submitted to midline laparotomy and 2 catheters insertion in bladder dome.

Only the animals in the first group were immediately sacrificed. In the four remaining groups the animals were kept alive for 7 days, with catheter exteriorization always in dorsal region near the animal head.

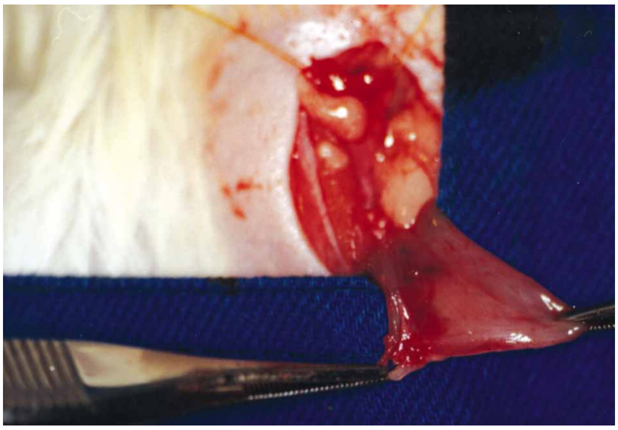


Figure 1 *– Myoperitoneal flap of the female rat rectus abdominis muscle.*

The group submitted to myoperitoneal cystoplasty, after this period, underwent a cystography (Figure-3), was sacrificed, and the augmented bladders were submitted to histological analysis. For all groups a measure of maximal bladder pressure and maximal bladder volume during urinary loss was performed. In group 1, cystometry was immediately done and in the remaining only at the 7th post-operative day. The equipment used in the study was model Dynapack MPX616, computerized – RS 232. An infusion pump, continuous type, with a 1mL syringe and 0.2mL/min saline infusion rate was also used.

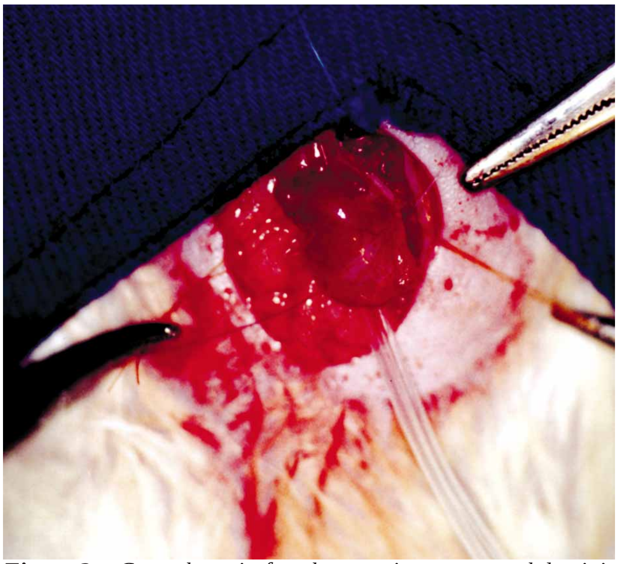


Figure 2 *– Cystoplasty in female rat using a rectus abdominis muscle flap. Two cystostomy catheters were used.*

Figure 3 *– Contrast X-ray, in a female rat, of a urinary bladder augmented with rectus abdominis muscle.*

The catheters were always 3F gauge with 20cm length made by non-reactive polypropylene. The suture used for the bladder was polyglycolic acid 6-0 (Vicryl™) and for the skin monofilament nylon 4-0.

Urinary bladder sent to histological study was fixed in formalin, processed in paraffin, sectioned, and stained with hematoxilin-eosin. Histological study was performed in the pathology department.

Variance analysis was used to verify means of maximal bladder pressure and maximal bladder volume during urinary loss equivalence among the groups studied (8).

Subsequently, Fisher's multiple comparison test was used to detect where are the differences between the groups studied (9), considering a global significance level $\geq$0.05 or 5%.

RESULTS

Maximal Bladder Pressure during Urinary Loss

Observing Table-1 and Figure-4 we may verify that, in all groups, the mean and the median were quite close, excepting for the group who under-

Table 1 – *Measures resume for Maximal Bladder Pressure in urinary loss (cm H_2O), for each type of surgery performed.*

	Type of Surgery				
Groups	**Immediate**	**Sham**	**Cystectomy**	**Cystoplasty**	**Cystostomy**
N	10	10	10	10	10
Mean	21.5	14.2	4.4	9.5	15.3
Standard-error	2.3	1.4	0.2	1.3	1.9
Median	21.5	13.0	4.5	8.0	15.0
Minimum	12.0	8.0	3.0	4.0	8.0
Maximum	32.0	20.0	5.0	18.0	23.0
Variation coefficient	0.3	0.3	0.2	0.4	0.4

went cystoplasty type surgery, and presented a diverse value for pressure measure.

When we analyze the variation coefficients of the study groups, we observe that the group submitted to partial cystectomy was the one presenting lower data variability, whilst the group submitted to immediate technique presented the greatest amplitude.

There are evidences that the mean of the group submitted to cystectomy type surgery was inferior to the mean of all the other groups, whilst the group submitted to immediate technique seems to have obtained the highest mean value of maximal bladder pressure during urinary loss (Figure-4).

Bladder Volume during Urinary Loss

Analyzing Table-2 and Figure-5 we may observe that each group presents very close mean and median values. The group submitted to cystectomy technique seems to have attained the lower mean value of bladder volume during urinary loss, whereas the group submitted to the cystoplasty technique seems to have attained the higher mean value.

Observing Figure-5 we note that, in the group submitted to technique sham, there was a possible “outlier” (discordant value).

When we verify the variation coefficient, we note that the group with greatest variability was the one submitted to the immediate technique, while the group submitted to cystectomy technique presented the lowest variation.

Inferential Analysis

Maximal Bladder Pressure during Urinary Loss

Observing the descriptive levels (Table-3), it is possible to note each surgical techniques presented significant differences. Maximal bladder pressure during urinary loss for the mean of study groups was lower in females rats submitted to cystoplasty compared to groups sham, immediate, and cystostomy; however it was higher than for the group submitted to partial cystectomy.

Bladder Volume during Urinary Loss

Observing the descriptive levels (Table-4) it is possible to verify which surgical techniques presented significant differences. Maximal bladder volume during urinary loss for the mean of study groups was higher in female rats submitted to cystoplasty compared to all analyzed groups.

Histopathological Analysis – Cystoplasty Group

Histological sections of the transition zone between the bladder and the muscular flap interposition demonstrated partial and/or total epithelization in the interspersed area of the muscular graft. The epithelium thickness in the graft region varied from two to six layers of transitional cells. In some cases, partial squamous metaplasia of the transitional epi-

Table 2 *– Measure resume for Bladder Volume in urinary loss (mL) for each type of surgery performed.*

	T ype of Surgery				
Groups	**Immediate**	**Sham**	**Cystectomy**	**Cystoplasty**	**Cystostomy**
N	10	10	10	10	10
Mean	0.168	0.195	0.112	0.281	0.200
Standard-error	0.014	0.007	0.003	0.018	0.008
Median	0.165	0.190	0.115	0.275	0.190
Minimum	0.100	0.180	0.100	0.200	0.180
Maximum	0.250	0.250	0.120	0.400	0.250
Variation coefficient	0.257	0.109	0.082	0.204	0.137

thelium was observed. In the original bladder wall a lining by the transitional epithelium of the smooth muscle tunic was observed, contrasting with the graft region showing lining by the same epithelium type, but presenting skeletal muscular tissue in the wall. The majority of cases demonstrated in the transition zone ulceration points covered by fibrin and neutrophiles, and chronic inflammatory process characterized by presence of lymphocytes and plasmocytes (Figure-6).

DISCUSSION

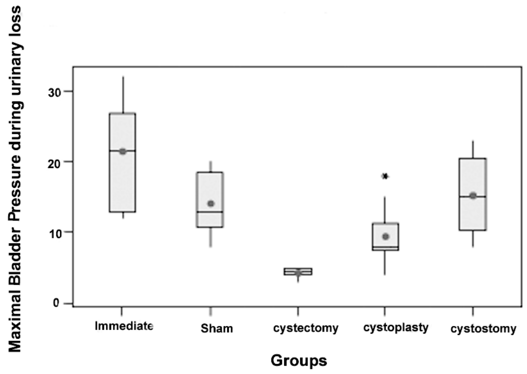
The search for an optimal tissue with the purpose of reconstructing the urinary bladder motivated publishing about several experimental and clinical essays.

Despite the progress, primarily in the use of detubularized gastrointestinal segments, one can recognize its limitations, especially concerning its complications (2,10). The study of new bladder reconstructive surgical alternatives requires experimental models to compare variables involved in the method used. It is wise to warrant the viability of an operative technique before its introduction in clinical practice. On the other hand, the severity of especial conditions may force the adoption of immediate and innovative solutions.

Skef et al. (1982) (11) performed surgical treatment in a 14 months child with bladder extrophy, using a segment of rectus abdominis muscle. The authors report the child developed hydronephrosis, requiring posterior ureteral reimplantation, yet they observed a good evolution of the grafted segment.

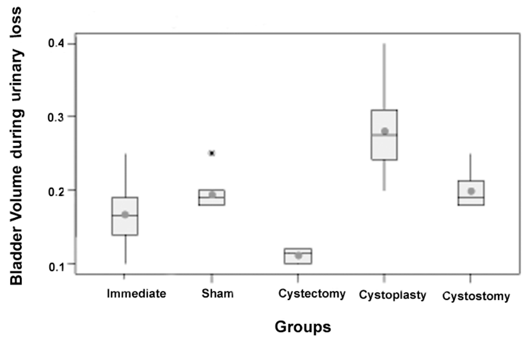
Table 3 *– Maximal Bladder Pressure during urinary loss. Descriptive levels of multiple comparisons by Fisher's method.*

	Surgical Technique				
	Immediate	**Sham**	**Cystectomy**	**Cystoplasty**	**Cystostomy**
Immediate	-	0.018	<0.001	<0.001	0.049
Sham	-	-	<0.001	0.026	0.640
Cystectomy	-	-	-	0.004	<0.001
Cystoplasty	-	-	-	-	0.021



Variance analysis: p<0,001. Fisher's multiple comparisons test: immediate > sham (p=0,018*), sham > cystoplasty (p=0,026*), immediate > cystectomy (p<0,001*), sham = cystostomy (p=0,640), immediate > cystoplasty (p<0,001*), cystectomy < cystoplasty (p=0,004*), immediate > cystostomy (p=0,049*), cystectomy < cystostomy (p<0,001*), sham > cystectomy (p<0,001*), cystoplasty < cystostomy (p=0,021*).*

***Figure 4** – Maximal Bladder Pressure in urinary loss (cm H_2O), for the groups analyzed.*



Variance analysis: p<0,001. Fisher's multiple comparisons test: immediate = sham (p=0,099), sham < cystoplasty (p<0,001*), immediate = cystostomy (p=0,067), sham = cystostomy (p=0,650), immediate > cystectomy (p=0,003*), cystectomy < cystoplasty (p<0,001*), immediate < cystoplasty (p<0,001*), cystectomy < cystostomy (p<0,001*), sham > cystectomy (p<0,001*), cystoplasty > cystostomy (p=0,002*).*

***Figure 5** – Bladder Volume in urinary loss (mL) for the groups analyzed.*

One should remember that, when this paper was published, little was known about bladder reconstruction with detubularized bowel segments in children.

Only after seven years, Büyükünal et al. (5) introduced in 1989 a pioneer experimental research, assessing the use of rectus abdominis muscle to bladder augmentation in rats. This study reported basically the possibility of grafted segment epithelization from the second day post-operative on. Besides this evaluation, the authors reported the possibility of a significant bladder capacity gain through cystographies analysis.

Motivated by the new bladder reconstruction alternative with peritonized rectus abdominis muscle, we opt to develop in our milieu an experimental study methodology, using female rats for a more detailed evaluation of this surgical technique, with an analysis of the urodynamics study for these animals. This evaluation would allow supporting this tissue viability to urinary bladder augmentation and its substitution, as well as the occasional functional consequence to the upper urinary tract.

In this case, aiming to define an efficient methodology, we developed an initial study, using ten female adult rats to standardize the urodynamics study

***Table 4** – Bladder Volume during urinary loss. Descriptive levels of multiple comparisons by Fisher's method.*

	Surgical Technique				
	Immediate	**Sham**	**Cystectomy**	**Cystoplasty**	**Cystostomy**
Immediate	-	0.099	0.003	<0.001	0.067
Sham	-	-	<0.001	<0.001	0.650
Cystectomy	-	-	-	<0.001	<0.001
Cystoplasty	-	-	-	-	0.002

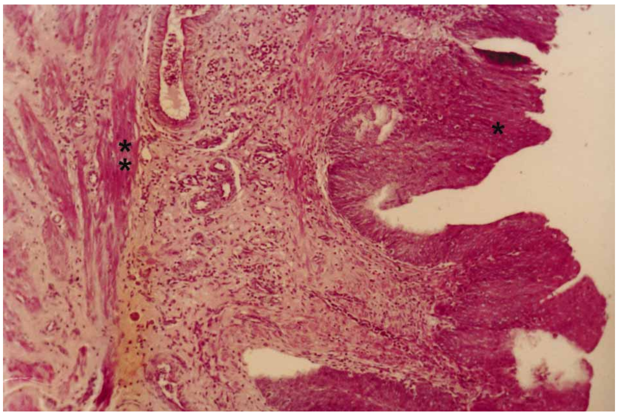


Figure 6 *– Photomicrography showing in higher magnification the lining by transitional epithelium (*) and presence of smooth muscle (**), HE, X160.*

performance in these animals and to the knowledge of the normal bladder histologic analysis (7).

Albeit urodynamics study is an important method to assess animals submitted to urinary reconstruction, we observed the existence of few experimental studies in rats capable to define urodynamics comparison parameters among different techniques.

Some authors tried to systemize the performance of the urodynamics study in normal animals.

Initially, Saito et al. (1996) (12), with the aim of studying regeneration and expansion of urinary tract in Sprague-Dawley rats, weighting 350 to 400g, performed bladder pressure study with suprapubic cystostomy, using a double lumen catheter and 0.05mL/min infusion rate.

The mean bladder pressures results in the control group were 29.8cm H_20, and bladder capacity was 0.4mL. The diversity of values found by these authors compared to our study, that showed lower values both in bladder capacity and pressure, is warranted by the use in this work of animals of higher weight than in our study (180 to 240g).

On the other hand, Tillig & Constantinou (1996) (13) developed an experimental method to investigate ureteral peristaltic dynamics. They have submitted 27 female rats to cystometric analysis, showing a bladder capacity similar to the one shown in our study, with mean maximal bladder pressure slightly superior to our data (mean=30cm H_2O).

The animals were submitted to inhaled anesthesia and the fluid infusion rate during cystometry was 0.2mL/min.

Regarding the method used to perform the cystometry we shall remind that in our work the animals were submitted to intraperitoneal anesthetics with thionembutal and that we used 2 suprapubic catheters aiming to reduce the interference of pressure transmission during fluid intravesical infusion. The infusion rate was also 0.2mL/min.

In other way, Miranda, D'Ancona et al. (1998) (14), also aiming to assess the use of muscle flaps in bladder reconstruction, performed a study in 24 animals, separating them in only two groups to analyze urodynamics parameters. This work used another urodynamics analysis methodology, for the rats were submitted to urethral catheterism to verify pressure measurements.

One of this methodology's embarrassments is that presence of a catheter in the urethra of the rats may present in fact a mechanism of infravesical obstruction, considering the diameter of this animals' urethra. All the same, the maintenance of a urethral catheter, or even via suprapubic, to posterior urodynamics analysis is not possible, since it is removed by the rats.

In our work we performed cystostomies with catheter exteriorization in rats' dorsal cervical region, making impossible its removal by the animal. The catheters assumed a subcutaneous route from bladder until the point of exteriorization.

Despite a distinct methodology, our study proved a bladder capacity gain (Figure-6) and epithelization of the neobladder for the mean of the animals evaluated.

Regarding histological analysis, our work demonstrated the epithelization of transitional cells in all the animals, varying from two to six layers, with absence of necrosis, indicating the good vitality of the muscular flap. The presence of an inflammatory process in most analysis possibly occurred due to local bacterial proliferation.

Moved by their experimental study, Büyükünal (15) published in 1994 his clinical experience in the surgical treatment of bladder extrophy in six patients using rectus abdominis muscle flap. The author pointed out in his work that despite the low number of operated children, there was technical viability in all cases, absence of mucus production, that prevents urinary obstruction and also absence of electrolytic disturbances by the epithelization of the interposed region by transitional cells.

Also, regarding the benefits of the technique, the author emphasized that it exempts manipulation of bowel loops, reducing surgical morbidity and the possibility of future malignization.

Despite his evaluation of bladder capacity and upper urinary tract consequences has been made solely by imaging exams, such as cystography and urography, the author did not present a control urodynamic analysis of the results in his study.

Our experimental work evidenced that rectus abdominis muscle interposition resulted in a mean gain of 44% in bladder capacity and a 37% decrease in both means compared to group sham. This data favors using this method, during maximal urinary loss pressure, knowing that probably a prejudicial pressure repercussion to upper urinary tract does not occur, with good preservation of renal function.

Our study allows stimulating the investigation about using rectus abdominis muscle in bladder reconstruction, but we should reinforce some limitations of this work regarding the application of this technique in future clinical series. The issue is the study is done with small animals, and bladder and rectus abdominis muscle dimensions are different than in humans. Standardization of urodynamics assessment in rats is preliminary and was done in a limited number of cases. In our experiment, data collection was performed in 7^{th} post-operative day. In this moment it was possible to obtain objective results consistent with our wishes, but follow-up time is short and its behavior during a longer follow-up is not known.

On the other hand, the study accomplished its objectives and serves as a reference to new experiments using rectus abdominalis muscle in bladder reconstruction. In this way we shall, in a subsequent study, evaluate the results of this technique in bigger sized animals.

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