

Single vs. double layer suturing method repair of the urethral plate in the rabbit model of hypospadias

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Introduction There are different methods of urethroplasty in hypospadias. The present study aimed to compare the repair of the urethral plate by single vs. double layer suturing.

Material and methods Fifteen male rabbits were assigned to the control, single layer, and double layer urethral plate suturing groups (n = 5). Experimental hypospadias was induced in the second and third groups and the urethral plates were sutured. After two weeks, the penis was dissected out and underwent histopathological processing. Stereological studies were applied to obtain quantitative histological data regarding the structure of the urethra and the related part of the corpus spongiosum.

Results Volume density of the urethral epithelium (the fraction of unit volume of the urethra occupied by its epithelium) was higher in the single layer suturing group when compared to the double layer or control groups (p < 0.01). Additionally, the volume density of the urethral lumen (the fraction of the corpus spongiosum that is occupied by the urethral lumen) in the single versus the double layer suturing groups was respectively 2.4 and 2 folds higher than that in the control group (p < 0.01). Besides, the volume density of the lumen was significantly higher in the single layer suturing when compared to the double layer suturing group (p < 0.01). However, no significant difference was observed among the study groups regarding the volume density of the collagen and vessels in the incised site of the penis which implied that the fraction of the urethra and surrounding corpus spongiosum was occupied by collagen and vessels.

Conclusions Urethral plate repair by the single layer suturing method could be accompanied by higher epithelialization and wider lumen in the rabbit model of hypospadias.

Key Words: urethra ↔ hypospadias ↔ urethroplasty ↔ rabbit ↔ stereology

INTRODUCTION

Hypospadias, as one of the most common congenital anomalies of external male genitalia, refers to an abnormal urethral opening where the urethra opens on the ventral surface of the penis [1–6]. Hypospadias affects approximately 1/125 to 1/300 live male births [1–6]. Up to now, several methods and innovations have been proposed to repair this abnormality, but

a fistula followed by dehiscence are the most common complications and side effects in almost all the cases [5]. In 1994, Warren Snodgrass described a procedure using a Tubularized Incised Plate (TIP) and reported its excellent results. In this method, making a longitudinal incision on the urethral plate with tubularization to make a neo-urethra and putting a flap on it could minimize the complications. The most important point in this technique

was making a relaxing incision in the urethral plate and using a protective dartos flap to cover the neo-urethra [6, 7]. After a TIP repair, fistulae were reported in up to 17% of the cases [8]. To our knowledge, in the TIP method described by Warren Snodgrass, urethral plate repair is done in two layers [9]. In some patients, on the other hand, the urethral plate is tiny and delicate which makes it difficult to be repaired in two layers. In such cases, this may tear the urethral plate and cause histological damage to the urethral plate tissue.

To the best of our knowledge, no studies have evaluated the difference between a single *vs.* double layer suturing method of urethral plate repair in a hypospadias model by stereological methods to obtain the quantitative histological data. Therefore, the present study aims to evaluate and compare the repaired urethra through single *vs.* double layer suturing in the male rabbit model of hypospadias and compare these two methods via quantitative stereological methods. Briefly, this study tries to answer the following question: Is there any difference between the urethral epithelium fractions after repairing the experimental hypospadias by single *versus* double layer suturing of the urethral plate? Is there any difference between the urethral lumen wideness after suturing the experimental hypospadias by single *versus* double layer suturing? Is there any difference between the collagen bundles and vessel density of the tissue after repairing by single *versus* double layer suturing?

MATERIAL AND METHODS

Animals

This study was conducted on fifteen male *Oryctolagus cuniculus* rabbits weighting 1600–2500 g. All the rabbits were kept individually and fed with standard food throughout the experiment. Each animal was kept in a cage with water and food *ad libitum*. The study was approved by the Research and Ethics Committee of Shiraz University of Medical Sciences. In addition, the animals were treated according to the guidelines for the care and handling of laboratory animals provided by Shiraz Laboratory Animals Center in accordance with the global standards for laboratory guidelines. The 15 rabbits were randomly allocated to three equal groups, namely single layer repair of urethra, double layer repair of urethra, and control groups, each containing 5 animals. According to standard stereological articles, 5 animals are sufficient in each group [10].

Urethral incision and repair

All the rabbits, except for those in the control group, were sedated using an intramuscular ketamine injection (10–15 mg/kg). Then, the external genital area was well shaved, prepared with a povidone-iodine topical antiseptic solution, and draped with sterile sheets. Under sterile conditions, the penis was exposed and a ureteral catheter (gauge 3) was inserted into the urethra. After catheterization, an incision was made on the ventral skin of the penis from the base to the glans over the midraphe line. The urethra was visualized and further excised over the catheter. Urethroplasty was done after incising the urethra. In one group, urethroplasty was done with suturing the urethra with Vicryl (7/0) in one layer, while in the other group it was done by suturing the urethra using Vicryl (7/0) in two layers. After closure, the catheter was removed and no dressing was applied, but an oxytetracycline spray was used instead for preventing infection of the wound. Finally, the rabbits in the control group were kept in standard laboratory conditions without undergoing any operation or urethral repair. After operating on the two groups, the rabbits were observed for bleeding, hematoma formation, and other complications daily for two weeks. After two weeks, all the penises were cut and deposited in buffered formaldehyde.

Stereological study

The body of each penis was cut from the basal part and the weight and volume were measured (according to the immersion method) [11–14]. In doing so, the penis was sectioned into slabs with equal distances. Then, ten to twelve transverse slabs of all parts of each penis were sampled according to the penis length. The sampled slabs of the penis were then processed and embedded in the same paraffin block. Overall, four micrometer sections were prepared and stained using Heidenhain's azan trichrome and hematoxylin and eosin. The microscopic evaluations were done using the computerized video-microscopy systems. In so doing, the stereological grid of points was generated using the software designed in the University and was projected on the live images of the sections.

Volume density (V_v) refers to the fraction of the unit volume of the tissue occupied by a structure [11–14]. The volume density of the urethral epithelium (the fraction of unit volume of the urethra occupied by its epithelium), and also volume density of the urethral lumen (the fraction of corpus spongiosum that is occupied by the urethral lumen) were estimated using the point counting method. Briefly, a grid of points

was superimposed on the live microscopic images of the urethral sections at the final magnification of 140 \times . In addition, the volume density of the collagen bundles and vessels in the incised site of the whole length of the penis was estimated (the fraction of the urethra and surrounding corpus spongiosum which is occupied by collagen bundles and vessels). To estimate aforementioned parameters, the grid of points was superimposed on the images of the tissues at the final magnification of 340 \times . The density was computed according to the following formula:

$$Vv(\text{structure, reference}) = P(\text{structure})/P(\text{reference})$$

Where 'P (structure)' was the number of points hitting the epithelium, lumen, collagen bundles, or ves-

sels and 'P (reference)' was the total number of points laid on the profiles of the urethra or the incised area.

Statistical analysis

The data was analyzed using the Kruskal-Wallis and Mann-Whitney U tests. Besides, $p < 0.05$ was considered to be statistically significant. The results were shown as standard dot plots.

RESULTS

As shown in Table 1, there were no significant differences between the animal weight, penis weight and penis volume in the single layer, double layer and the control group.

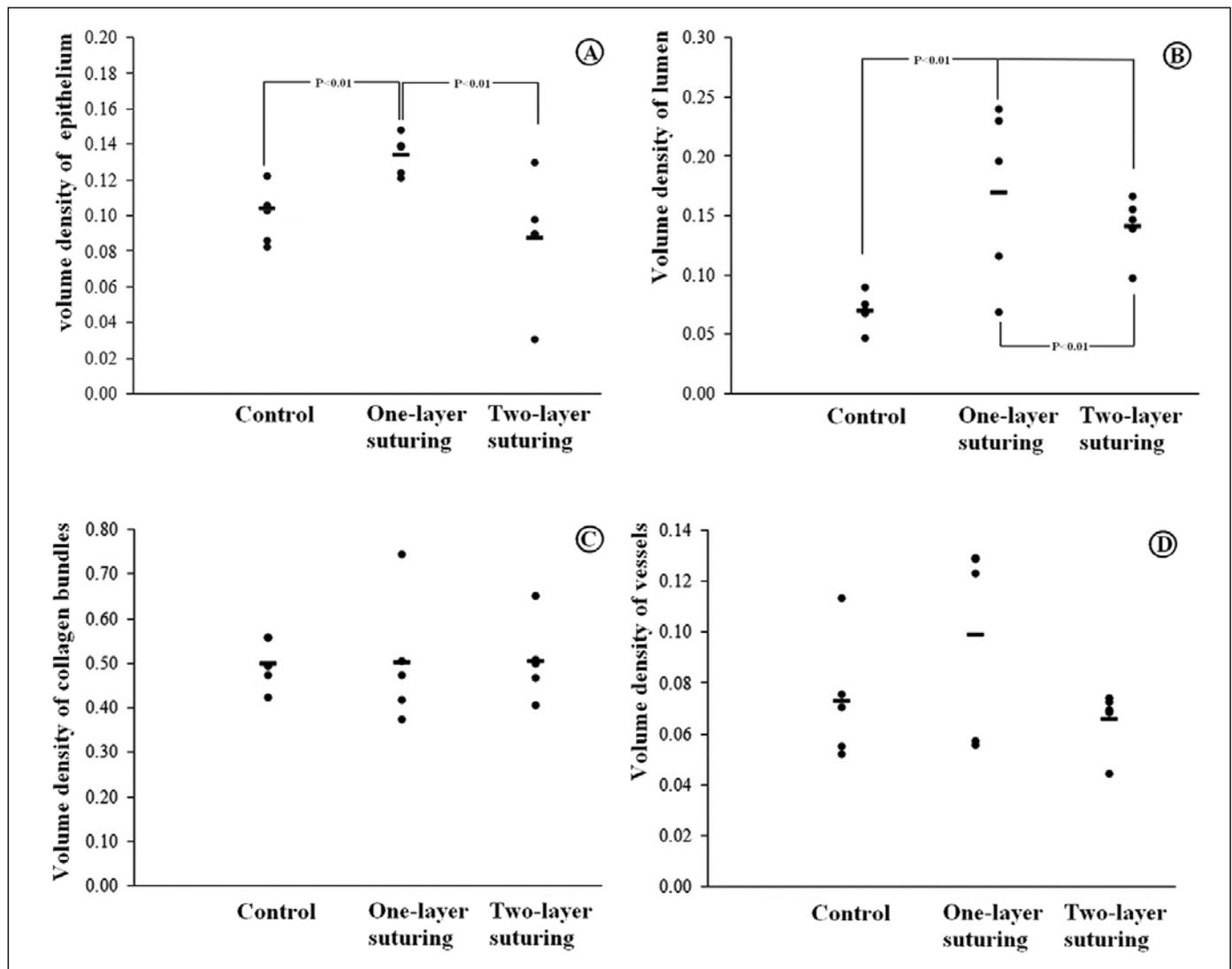


Figure 1. The dot plots of the volume density of the urethral epithelium (A), urethral lumen (B), collagen bundle (C), and vessels (D) in the incised site of the penis just inferior to the urethra down to dermis of the incised area in the control, hypospadias+single layer suturing, and hypospadias+double layer suturing groups. The differences among the groups have been indicated on the plots.

Table 1. Mean animal weight (g), penis weight (mg) and penis volume (mm³) of the body of the penis of rabbits in the three animal groups including the control, single layer, and double layer

	Control	Single layer	Double layer
Animal weight	1990	2240	2020
Penis weight	470	464	410
Penis volume	453	440	389

Volume density of the urethral epithelium was higher in the single layer suturing group when compared to the control group ($p < 0.01$), but no significant difference was found between the double layer suturing and control groups in this regard. Volume density of the urethral epithelium was also increased in the single layer suturing group in comparison to the double layer suturing group ($p < 0.01$) (Figures 1 and 2).

Volume density of the lumen in the single when compared to the double layer suturing groups was respectively 2.4 and 2 folds higher than that in the control group ($p < 0.01$). Additionally, the volume density of the lumen was significantly higher in the single layer suturing group in comparison to the double layer suturing group ($p < 0.01$) (Figures 1 and 2).

However, the results showed no significant differences between the single layer suturing, double layer suturing and the control groups regarding the volume density of the collagen and vessels. Also, no significant difference was observed between the two suturing methods concerning these parameters (Figure 1). No sign of fistula was seen during the histological study of the penis.

DISCUSSION

Hypospadias, as one of the most common congenital anomalies of external male genitalia, refers to an abnormal urethral opening where the urethra opens on the ventral surface of the penis [1, 2]. Up to now, many different hypospadias repair methods have been developed by urologists around the world. Among these techniques, the TIP method developed by Warren T. Snodgrass has been considered to be the best method for urethral repair in hypospadias [15]. New methods of hypospadias repair try to repair the penis both functionally and cosmetically [6]. However, there are some complications for primary hypospadias repair. Urethrocutaneous fistula is the most common complication of hypospadias repair and occurs at various rates [9, 16–19]. Appignani et al. [20] reported that the double-dartos flaps had significantly lower rates of fistula formation. Most studies conducted on the issue, including those by Yigiter et al. [21], Elsayed et al. [22], and Erol et al. [23], also indicated lower rates of fistula formation using double-dartos flaps, but the difference was not statistically significant. Bertozzi et al. [24] performed a multicenter review of 394 cases of hypospadias repair covered with double-dartos flaps and reported fistulae in only four cases [1.01%]. Besides, all the fistulae were small and healed spontaneously after a few weeks. Djordjevic et al. [25, 26] also reported no fistula formation with single-dartos flaps. However, other researchers did not get similar results and reported fistula rates of 13% [27] and 26% [22] using single-dartos flaps. As mentioned above, the most common complication of hypospadias repair is fistula, which can be prevented by placing a barrier layer between the neourethra and the overlying glans and shaft skin closures.



Figure 2. The microscopic photomicrograph of the urethra in the in the control (A), hypospadias+single layer suturing (B), and hypospadias+double layer suturing (C) groups. The asterisks indicate the urethral lumen. The lumen is wider in the single layer suturing group compared to the other groups. Surrounding the urethral epithelium is also more abundant in the single layer sutured animals.

This coverage is best achieved by a de-epithelialized dartos flap layer [6]. The second most common complication of hypospadias repair is meatal stenosis, which may result from some technical errors. Other complications include urethral stricture, diverticulum, and wound dehiscence [6]. In some cases, the urethral plate is very thin and may be torn if repaired in two layers. Also, two-layer suturing may cause histological damages to the urethral tissue. However, the present study focused on the quantitative methods to present a reliable conclusion. The data showed that a higher fraction of the urethra was occupied by the urethral epithelium when it was repaired using the single layer suturing. Moreover, a higher fraction of the penis was occupied by the urethral lumen in the single layer suturing. However, no significant difference was found among the study groups regarding the volume fraction of the collagen and vessels. The higher volume density of the urethral epithelium and lumen in the single layer method in our study might be due to the lower suture tension on the tissue, leading to better results and lower complications. Yet, this should be proved in future studies. Although we have no explanations for the more luminal epithelium in the single layer suturing group, the significant increase in the luminal epithelium is important and valuable in the process of wound healing. This indicates the epithelialization of the urethra. Epithelialization is defined as the process of covering the denuded epithelial surface. The cellular and molecular processes involved in initiation, maintenance, and completion of epithelialization are essential for a successful wound closure. In fact, epithelialization is an essential component of wound healing and is used as a defining parameter of its success. In the absence of re-epithelialization, a wound cannot be considered to be healed. Upon acute skin injury, as the barrier is disrupted, neutrophils, monocytes, and macrophages enter the site of injury. Subsequently the activation process is achieved by expression of several cytokines and growth factors. The activated phenotype is marked by changes in the cytoskel-

eton network and cell surface receptors essential for re-epithelialization [28].

This study had some limitations. First of all, this was an animal study and its clinical aspects should be documented in the future. Secondly, the animals that underwent the study had normal urethra and they did not portray exactly the abnormal urethral plate in real cases. Furthermore, it might be better to incise the urethra in one session, leave it for a while and then repair the urethral plate in the following session. Thirdly, the fistula rate was not evaluated in this study. The radiological imaging might be advised to compare the rate of fistula between these methods in a future study. Moreover, these results might not be applicable to redo cases, due to the scarred and fibrotic plate which may change the histological pattern.

Overall, the current study findings revealed that repairing the urethra in two layers was not an obligation and considering the stereological evaluation of the two groups, single layer repairing seemed to be more applicable. In cases with thin and tiny urethral plates, single layer repairing is easier to do and does not lead to tearing of the urethra. Nonetheless, these results were obtained from an animal study and, consequently, further studies have to be performed on human models to evaluate the efficacy and complications of the procedure.

CONCLUSIONS

Urethroplasty by the single layer suturing method could be accompanied by higher epithelialization and wider lumen in the rabbit model of hypospadias.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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