

ASSESSMENT OF USING BIOLOGIC SURGICAL ADHESIVE (BIOGLUE) ON PREVENTING URINE LEAKAGE AFTER SURGICAL REPAIR OF HYPOSPADIAS IN ANIMAL DOG MODEL

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ABSTRACT

Aim in this study was to evaluate the protective effect of Bioglue for suture line in the repair of hypospadias.

Back ground: Bioglue has been used in the treatment of many surgical diseases. The main purpose of Bioglue was as protective layer for traditional suture line or replacement of traditional suture line. Different studies evaluated different methods and techniques to reduce urethra-cutaneous fistula which is a major complication of hypospadias repair. But review of literatures reveals that they were not successful completely.

Materials and Methods: 16 dogs (4 to 6kg German Shepherd) enrolled in this study in animal lab of Shiraz University of Medical Sciences. Dogs were divided into two groups. Group I repaired with a layer of Bioglue between the protecting flaps and Group II without it; repair performed in fine manner with PDS 6-0 in both groups. One case and one control dog were sacrificed after two weeks then the remaining sacrificed in two weeks intervals sequentially. Urethra was checked grossly and radiological for leakage. Then penis harvested and underwent histopathologic evaluation with light microscope by Hematoxylin and Eosin staining.

Results: Two out of eight (25 %) urine leakage confirmed radiological and grossly in GII versus no leak in GI (Predictive Value of 0.23). There were No evidence of ossification, stenosis, stricture or increased collagen formation in both groups (II, I) and all tissue healing process were same.

Conclusion: We recommend using Bioglue in repair of hypospadias in animal in further larger studies to re-evaluate its effect on protection of suture line. If future studies confirm its role in animals, we can use it not only as a sealant layer but also as scaffold that helps better wound healing for prevention of fistula formation in human hypospadias repair.

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INTRODUCTION

Hypospadias is an abnormality of anterior urethral development in which the urethral opening is ectopically located on the ventrum of the penis proximal to the tip of the glans penis (Baskin, 2000).

The location of the abnormal urethral meatus classifies the hypospadias locations include anterior middle and posterior. Sub coronal type is the most common (Barcat, 1973 and Duckett, 2002). It is a common developmental disorder of the urogenital tract, occurring in approximately 1 in 125 live male births (Stokowski, 2004). Hypospadias is often associated with a deficient prepuce and chordee. It is usually occurs as an

isolated defect, but can be part of a recognized syndrome or associated with other genital anomalies such as undescended testes, cryptorchidism and inguinal hernia (Stokowski, 2004). Currently, most physicians attempt to repair hypospadias when the child is aged 6-18 months (Dodson, 2007). Treatment is based on surgical intervention and has different methods based on type of hypospadias (Chen, 2000; Cook, 2005). The goals of treating hypospadias are to create a straight penis by repairing any chordee (orthoplasty), to create a urethra with its meatus at the tip of the penis (urethroplasty), to re-form the glans into a more natural conical configuration (glansplasty), to achieve cosmetically acceptable penile skin coverage, and to create a normal-appearing scrotum. The resulting penis should be suitable for future sexual intercourse, should enable the patient to void while standing, and should present an acceptable cosmetic appearance (Snodgrass, 2007). Urethrocutaneous fistulisation is a major concern in hypospadias repair (Snodgrass, 1999). The rates of fistula formation generally are 10%-40% and are repaired using a multi-layered closure with local skin flaps. Still, fistulas may recur in approximately 10% of cases. Urine diversion and prevention of overlapping suture line, two stage repairs, dartose flap and tunica vaginalis interposition layer (HandooYogRaj, 2006 and Wen-Chou Lin, 1990). Bioglue is a two-component surgical adhesive composed of purified bovine serum albumin (BSA) and glutaraldehyde (CryoLife Incorporation, 2001). Bioglue has been used successfully for air leakage in lung operation (Tansley, 2006), CSF leakage (Kumar, 2003), heart, fistulas repair and microvascular anastomosis (Xiaohong Wang, 2004), and urine leakage in prostatectomy (Hruby, 2006).

History of Hypospadias

Hypospadias, or atypical placement of the urethral meatus, is a common finding during routine examination of the new-born. The term hypospadias comes from the Greek words hypo, meaning below, and spadon, meaning opening (Bukowski, 2001). First described in the second century by *Galen* in the second century A.D., who gave the condition its name, then almost forgotten until *Mettauer* in 1842, all previous surgeons overstressing the position of the orifice. *Mettauer* recognised skin shortening as a cause of chordee, a fact not re-discovered until 1967. Urethroplasty from penile skin in situ was well described by *Thiersch* in 1869 and *Duplay* in 1874; additional covering skin flaps were developed in 1892 by *Lauenstein*.

Penile Embryology

By the end of month 1 of gestation the hindgut and future urogenital system reach the surface of the embryo at the cloacal membrane on the ventral surface. The cloacal membrane is divided by the urorectal septum into a posterior or anal half and an anterior half called the urogenital membrane. Three protuberances appear around the latter structure. The most cephalad protuberance is the genital tubercle. Masculinization of the external genitalia occurs under the influence of testosterone in response to a surge of luteinizing hormone from the pituitary gland. An initial sign of masculinization is an increase in the distance between the anus and genital structures, followed by elongation of the phallus, formation of the penile urethra from the urethral groove and development (Baskin, 2000). At 8 weeks of gestation the external genitalia remain in the indifferent stage (Fig. 2-1. A).

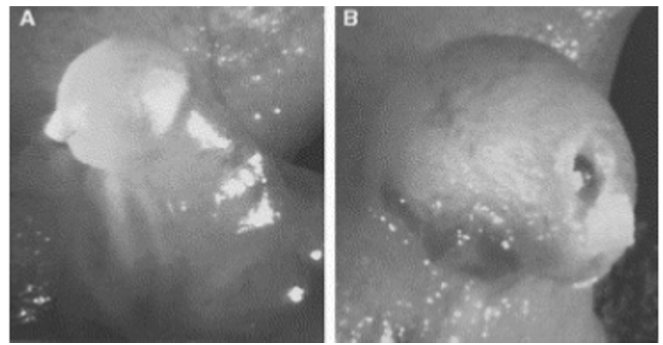


Fig. 2.1. Male genitalia during gestation

- At 10 week urethra is open and urethral folds are prominent
- At 16 weeks penile and urethral development is complete with prepuce covering glans

Incidence

The incidence of hypospadias is about 1 in every 300 male children. If minor degrees of hypospadias are included, this incidence may be as high as 1 in 125 male births (Duckett, 1989). It has been estimated to be between 0.8 and 8.2 per 1000 live male births (Sweet, 1974). The risk of occurrence in an infant is 8% if his father has hypospadias and 14% if a sibling has the condition; if two members of the same family have hypospadias, the risk is about 21% (Duckett, 1989). In several countries, the incidence of hypospadias may be rising but seems rather constant at 0.26 per 1000 live births in Mexico and Scandinavia and 2.11 per 1000 live births in Hungary (Kallen, 1983). In Europe the prevalence of hypospadias in the 1970's and 1980's has been increasing with no obvious explanation. In the United States data from two birth defects surveillance systems has also shown an unexplained doubling in the incidence of hypospadias (Paulozzi, 1997). The U.S. study from the Centre of Disease Control is particularly intriguing in that the incidence of severe hypospadias, not just mild forms, is increasing implying that the increase in hypospadias is not secondary to an increase in surveillance or reporting (Baskin, 2000).

Etiology

Understanding of the embryologic development of the penis confirms that hypospadias results from partial or complete failure of urethral folds to form throughout their normal length. Hypospadias also may result if urethral folds that have formed fail to close distally; the extent of the closure determines the position of the urethral orifice (Shukla, 2004). A unifying etiology for hypospadias remains elusive and is likely multifactorial (Shukla, 2004). It is probably polygenic because of the higher familial incidence. The condition is more common in white than in black individuals and most common in Italian and Jewish individuals (Welch, 1979). The incidence of hypospadias in monozygotic twins is 8.5-fold that in singletons (Roberts, 1973). It is also possible that for monozygotic twins 1 placenta may not meet the HCG requirements for masculinization of 2 male fetuses. A more reasonable theory to explain hypospadias is an abnormality in androgen metabolism. *Allera et al* evaluated 9 patients with severe hypospadias and detected a defect in open reading frame of the androgen receptor in only 1 (Allera, 1995). Sutherland et al also concluded that mutations in the androgen receptor gene are rarely associated with hypospadias (Sutherland, 1966).

Genes and Hypospadias

Normal sexual differentiation depends on testosterone and its metabolites as well as a functional androgen receptor. Genetic defects in the androgen metabolism pathway, that is 5 α -reductase defects or androgen receptor defects, are known to result in hypospadias. Possible other candidates include genes known to have a role in limb development during embryogenesis (Lindsey, 1996; Mark, 1997 and Geraudie, 1998).

Endocrine Disruptors

A possible explanation of the worldwide increase in the incidence of hypospadias may be environmental contamination. In this regard it is well established that humans continually ingest substances with known estrogenic activity, such as insecticides used in crop production, natural plant estrogens, by products of plastic production, pharmaceuticals and so forth.

ASSOCIATED FINDINGS

Cryptorchidism and inguinal hernia

Between 8% to 10% of boys with hypospadias have a cryptorchid testicle and 9% to 15% have an associated inguinal hernia (Khuri, 1981; Sweet, 1974 and Sorber, 1997). In boys with more proximal hypospadias, cryptorchidism may occur as frequently as 32% (Cerasaro, 1986). The incidence of chromosomal anomaly in these groups of patients is much higher (22%) than hypospadias (5%–7%) or cryptorchidism (3%–6%) occurring alone (Yamaguchi, 1991; Moreno-Garcia, 2002).

Intersexuality

Hypospadias and intersexuality also are believed to represent two ends of a spectrum. The more severe the hypospadias, the more likely an intersex state exists (Kaefer, 1999). *Rajfer and Walsh* (Rajfer, 1976) reported intersexuality in 27.3% of boys with a normal-sized phallus, cryptorchidism, and hypospadias. Partial androgen insensitivity, chromosomal abnormalities, *Smith-Lemli-Opitz syndrome*, 5 α -reductase deficiency, *Drash syndrome*, and other conditions also can occur in association with hypospadias (Shukla 2004).

Presentation

The anatomic location of the meatus and extent of ventral curvature, or chordee, should be determined. In some instances, multiple pinpoint dimples may be present on the surface of the urethral plate in addition to the hypospadias urethral meatus. Meatal position may be classified as anterior (distal), middle, and posterior (proximal), with more anatomically specific subgroups being applied further (Fig. 2-7). The meatus is located on the glans or distal shaft of the penis in approximately 70% to 80% of boys with hypospadias. The meatus is in the middle of the shaft of the penis in 20% to 30% of boys with hypospadias (Shukla, 2004). Increased understanding of the endocrinological origins of hypospadias has corroborated the clinical association of hypospadias with intersex states (Rajfer, 1976 and Aarskog, 1970).

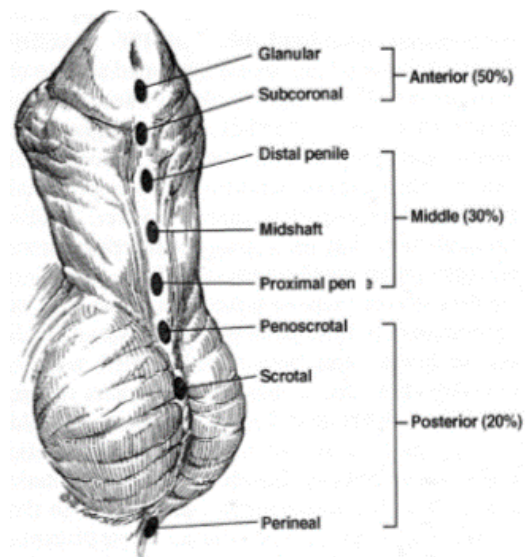


Fig. 2.7. Classification of hypospadias based on anatomic location of the urethral meatus. Anterior, or distal hypospadias is the most commonly encountered variant

Koff and Jayanthi (Koff, 1999) showed that androgenic pre-treatment with hCG may increase penile length and move the meatus to a relatively more distal position as the shaft elongates in response to the hCG.

Surgical Repair

The goal of hypospadias surgery is a functional sexual organ that is free of curvature. Equally important is a glanular urethral meatus that allows a boy to void with a laminar flow while standing (Shukla, 2004).

Timing of Surgery

Experience over the past decade confirmed the American Academy of Pediatrics' review that the ideal age for genital surgery is between 6 to 12 months of age.

Hemostasis

Adequate haemostasis may be achieved by various techniques in hypospadias surgery. Some surgeons place at the base of the penis a tourniquet that is removed every 45 minutes alone or combined with needlepoint spot and bipolar electrocoagulation to control blood loss that may obscure the surgical field. The authors minimize the use of electrocautery to reduce the potential for tissue damage from cautery dispersal and instead inject 1:100000 epinephrine in 1% lidocaine along the proposed incision line. In the authors' experience, the epinephrine provides adequate local hemostasis and the injection separates the skin and dartos that facilitates the initial dissection (Shukla, 2004).

Types of Hypospadias Repairs

A plethora of surgical options, from novel approaches to modifications of known procedures, have been described for various presentations of hypospadias. The surgical technique that is most appropriate for a given case is predicated heavily on anatomic factors, previous surgical descriptions, and a surgeon's personal experience (Shukla, 2004).

- Distal Hypospadias Meatal advancement, glanuloplasty (MAGPI)
- Tubularized, incised plate urethroplasty
- Middle and Proximal Hypospadias or Distal Hypospadias with Chordee.

Island onlay hypospadias repair

Transverse island tube repair

Two-stage repair

Free grafts

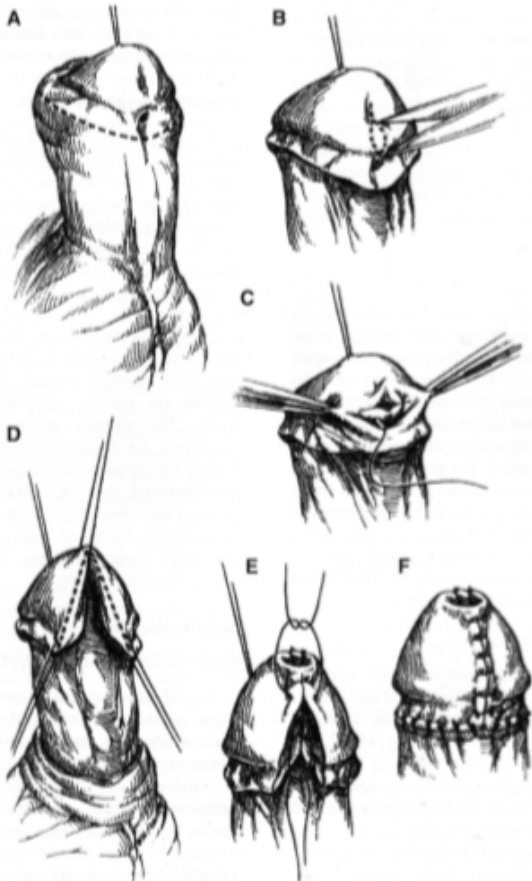


Fig. 2.8. Meatoplasty and glanuloplasty repair.

- (A) Circumferential incision 5 cm below the corona. (B) Transverse bridge of tissue distal to the meatus is incised sharply. (C) A Heinek-Milulicz closure opens and advances the meatus. (D) Ventral meatal edge is pulled distally, and the exposed glans edges are trimmed and approximated. (E) Glansplasty is performed with subepithelial sutures to leave a rounded, conical glans. (F) Dorsal skin is transferred ventrally; excess skin is excised, and no catheter is left in place

Complications

Urethrocutaneous fistula

Salvage hypospadias repair

Bioglue Surgical Adhesive

Description

Bioglue Surgical Adhesive (Bioglue) is a two-component surgical adhesive composed of purified bovine serum albumin (BSA) and glutaraldehyde. The BSA is obtained from cattle exclusively from bovine spongiform encephalopathy (BSE) free countries and undergoes processing that reduces or inactivates viruses. Bioglue begins to polymerize within 20 to 30 seconds and reaches its bonding strength within 2 minutes. Bioglue also adheres to synthetic graft materials via

mechanical interlocks within the interstices of the graft matrix. The Bioglue component has a shelf life of 3 years if stored at 25 °C (15).

Indications for Use

Bioglue Surgical Adhesive is indicated for use as an adjunct to standard methods of achieving hemostasis (such as sutures and staples) in adult patients in open surgical repair of large vessels (such as aorta, femoral, and carotid arteries) (CryoLife Incorporation, 2001). Also, it has been used for anastomosis of small arteries based on soluble stent (glucose, dextran-40 and disodium (Baskin, 2000 and Stokowski, 2004)-2-deoxy-2-sulfoamino-β-Dglucopyranuronan (S-chitosan with Bioglue in an outer layer (Economopoulos, 2004). Bioglue has been used successfully for air leakage in lung operation, postoperative airleak management (Handoo Yog Raj, 2006).

Contraindications

- Not for patients with a known sensitivity to materials of bovine origin
- Not for intravascular use
- Not for cerebrovascular repair

Observed Adverse Events

Adverse events observed during the clinical studies included the following:

2-15-6. Observed Adverse Events

Adverse events observed during the clinical studies included the following:

• Bioglue applied to non-targeted tissue	• Neurological deficit
• Failure of Bioglue to adhere	• Organ system failure
• Death	• Paraplegia
• Hemorrhage	• Pleural effusion
• Infection	• Renal dysfunction/failure
• Inflammatory, immune systemic allergic reaction	• Irreversible morbidity
• Respiratory dysfunction/failure	• Ischemia
• Stroke or cerebral infarction	• Myocardial infarction
• Thromboembolism	
• Thrombus	

Potential Adverse Events

- A hypersensitivity reaction such as swelling or edema at the application site
- Application of adhesive to tissue not targeted for procedure
- Failure of Bioglue to adhere to tissue
- Local tissue necrosis
- Mineralization of tissue
- Adhesions
- Neurological deficits
- Possible transmission of infectious agents from material of animal origin
- Thrombosis and thromboembolism
- Anastomotic pseudoaneurysm
- Organ system dysfunction/failure
- Aortic insufficiency
- Paraplegia
- Cardiac tamponade
- Pleural effusion
- Cerebral emboli
- Pulmonary emboli
- Death or irreversible morbidity

- Dissection
- Injury to normal vessels or tissue
- Vasospasm
- Vessel rupture and hemorrhage (CryoLife Incorporation, 2014)
- SVC Stenosis (Economopoulos, 2004)

MATERIALS AND METHODS

The study was conducted in sixteen dogs (4 – 6 kg), German Shepherd, for creation of hypospadias. They were divided into two groups, group I (case) and group II (control). Each group included eight dogs.

This study was performed in animal research laboratory following permission from medical research and veterinary ethic's group. All steps and care of dogs during study also were compatible with National health and medical research council (NHMRC) ethical policy for dog studies (National Health and Medical Research Council, 2004).

Procedure

Anesthesia was done by Ketamine (10 mg /kg) and diazepam (1mg/kg) via cephalic root and IV antibiotic penicillin benzathine (22000 IU/ kg) was administered 1 hour before incision.

Hypospadias creation: dogs all prepped and dreped in the supine position by bethadine solution. French catheter number 6 inserted into the urethra and urine withdrawn for proper position confirmation. Penis incised in both group in the midshaft portion with the length of 2.5 cm from skin up to the urethral mucosa. During this procedure injury to the carpus spongiosus avoided delicately.

Repair: The urethral defect in group I was repaired by one layer of continuous PDS 6/0 (ETHICON, 3/8 Circle, 10.5mm, Violet Monofilament, Product Code Z489G) suture, followed by thin coat (1 mm thick) of Bioglue that was composed of purified bovine serum albumin (BSA) and glutaraldehyde and was supported by two layers of dartos and prepuce. The defect in group II was closed in two separate layers of PDS 6/0 continuous suture which supported by the same layers of dartos and prepuce. Then urinary catheter fixed into the glans with stitches and antibiotic (OTC tetracycline veterinary type) soaked dressing was applied. For prevention of dressing avulsion by dogs they were fixed with four stitches into the abdominal wall. Dressings were removed after 48 hours (time of usual dressing removal because of concordance with 50 epithelialisation completion time. Then urinary catheter discontinued the day After.

Dogs were sacrificed every two weeks in both groups (after two, four, six and eight weeks in each episode two dogs were sacrificed, one from case and another from control group). We sacrificed dogs by IV administration of KCL 40 % (4 cc/ kg). Urethra was checked for leakage after saline injection grossly and by retrograde urethrogram (RUG) in radiologic ward by cross table X-ray. We assessed slides for different type of cellular infiltration in different phases of study (2, 4, 6 and 8 wks), level of collagen formation and tissue fibrosis, presence or absence of orthotopic and heterotopic ossification.

RESULTS

Two out of eight (25 %) urine leakages confirmed radiologically and grossly in group II versus no leak in group I ($P 0.23$) all urethra was patent in both groups, patency assessed by Retrograde Urethrogram. We did not have any evidence of stenosis of urethra. In Both groups, pathologic examination with "H & E" staining and collagen specific dye under light microscope revealed same level of cellular infiltration in both groups and there was not any evidence of over-fibrosis or ossification changes in Bioglue group in our study.

All data were analysed with one sided fisher's exact test by SPSS version 13 program: Study did not show any statistical evidence of Bioglue support layer role in prevention of further urine leakage or fistula formation in hypospadias repair. Besides, it did not show any evidence of statistical significant or clinical important side effects of Bioglue including stenosis, stricture, hetro or orthotopic ossification of the urethral tissue.

In pathologic examination we did not see any possible cytotoxic or cytoidal effect of glutaraldehyde component of Bioglue in different infiltrative process of wound healing.

Table 4.1. Statistical comparison of hypospadias repair complications in case and control groups. The same dogs with gross leak had radiological leak

Groups	Control Group	Bioglue Group	P value
Clinical leak	2(25%)	0(0%)	0.23
Radiological leak	2(25%)	0(0%)	0.23
Clinical stricture	0(0%)	0(0%)	NS
Radiological stricture	0(0%)	0(0%)	NS
Pathological stricture	0(0%)	0(0%)	NS
Infection	0(0%)	0(0%)	NS
Mortality	0(0%)	0(0%)	NS
Fibrosis	0(0%)	0(0%)	NS
Ossification	0(0%)	0(0%)	NS

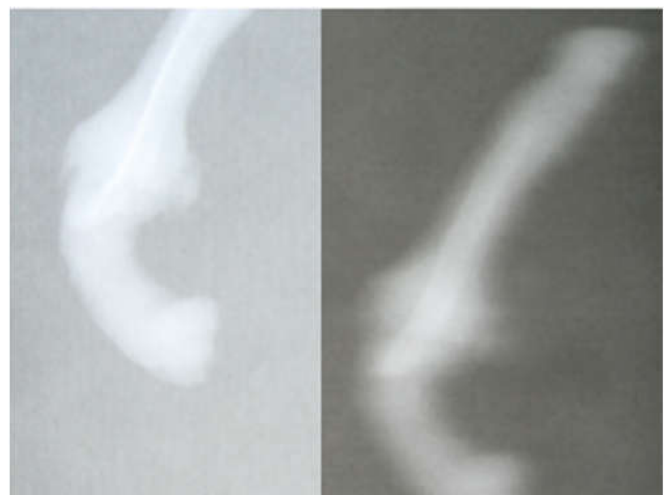


Fig. 4.1. Urethrogram of case and control after two weeks of study as you see in this picture left sided image which is case is not different with control group in respect of patency but some amount of extravasation as a leak seen in the control group



Fig. 4.2. After weeks of repair left side case and right side control

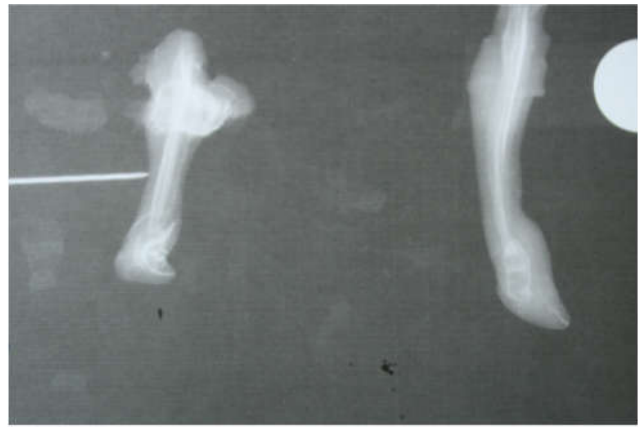


Fig. 4.3. After eight weeks of study both group urethra are patent as showed in this image left side case group and right side control



Fig. 4.4. Cross section of urethra in control group with H&E Staining Shows patency and normal architecture

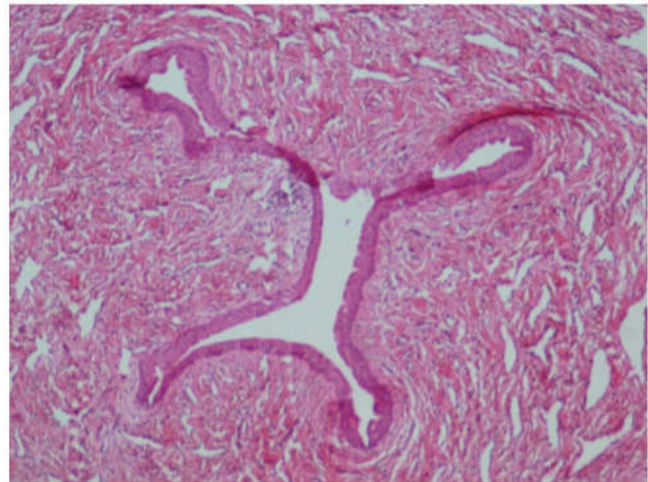


Fig. 4.5. Cross section of urethra in case group shows the same level of infiltration and also the same type of cells in comparison to control group

DISCUSSION AND CONCLUSION

Urethrocutaneous fistula is the most common complication of hypospadias surgery (Snodgrass, 1999). Different studies demonstrated different rate of fistula formation and recurrence based on the method of repair. *Smith D.* in a study of hypospadias surgery noted varied fistula rates for different procedures. Flip-flap repair varied from 2.2 to 35%, island pedicle tube flap 4 to 35%, free graft tube flap had a fistula rate of 15 to 50% (Smith, 1981). *Retik et al* used the dorsal dartos as a covering layer between the neourethra and the penile skin to avoid the occurrence of urethrocutaneous fistula. However, the dorsal prepuce tends to suffer from blood supply deterioration when aggressive dissection between the skin and the dorsal dartos is conducted to provide the abundant dartos flap (Retik, 1995). *Belman et al* wrapped the neourethra with a deepithelialized preputial skin flap which was rotated to the ventrum from the dorsal aspect and the incidence of fistula after the hypospadias repair was only 3.5%. *Snow et al.* developed a novel technique for wrapping a long neourethra for correcting proximal hypospadias. Tunica vaginalis has sufficient vascularity and a large amount of tissue to cover the neourethra of proximal hypospadias, as well as distal ones, without difficulty. They performed the tunica vaginalis blanket technique without occurrence of fistula in 20 patients (Snow, 1995).

Severe hypospadias tends to be associated with bifid scrotum or penoscrotal transposition. Therefore, dissection inside the scrotum could be automatically accomplished at the step of corpoplasty and urethroplasty and we can easily obtain abundant dartos tissue for wrapping the long neourethra. While a testis must be exposed to gain the tunica vaginalis with Snow's technique, we avoided the exposure by separating the dartos fascia and the tunica vaginalis containing a testis using Churchill's technique without opening the tunica vaginalis (Churchill, 1996). One of the most important concerns about Bioglue usage is tissue toxic effect of glutaraldehyde and delayed ossification like reactions. *Fürst W et al*, in one invitro study, assessed cytotoxic effect of Bioglue by adding the supernatants to either cultured human embryo fibroblasts (MRC5) or mouse myoblasts. *Economopoulos GC et al* in another study showed late effect of Bioglue as fibrotic tissue production resulting in stenosis of superior vena cava (SVC) when applied there (Economopoulos, 2004). According to literature review our study is the first one for assessment of Bioglue role in the repair of hypospadias. It did not increase risk of infection nor locally or systematically. Pathologic reports did not show increase in tissue fibrosis or abnormal tissue ossification (nor orthotopically or heterotopically). Bioglue reduces risk of urethrocutaneous fistula formation clinically but did not increase risk of urethral stenosis either clinically or retrograde urethrographically documented.

Persistency of Bioglue effect in our study was for more than eight weeks and in other studies up to three years reported (CryoLife Incorporation, 2001). Besides, statistically our result did not prove sealing effect of Bioglue in the repair of hypospadias (Table 4-1). Possible cause for this statistic may be shortage of sample.

Conclusion

We recommend using of Bioglue in repair of hypospadias not only as a sealant layer but also as scaffold that helps better wound healing for prevention of fistula formation and, we should consider animal studies in larger scale and possible future clinical trials in human. We should try to bring out more easily applicable Bioglue guns with fewer prices.

Disclosure: The authors declare no conflicts of interest.

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