The use of autologous skeletal muscle - derived cells as a sling in the treatment of stress-induced urinary incontinence : an experimental study in dogs

Bassem S Wadie (bassem_wadie@mans.edu.eg)
Mansoura University Urology and Nephrology Center https://orcid.org/0000-0002-6977-6849
Haytham G Amer
Mansoura University Urology and Nephrology Center
Sheryy M Khater
Mansoura University Urology and Nephrology Center
Mahmoud M Gabr
Mansoura University Urology and Nephrology Center

Research Article

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Abstract

Purpose:

This is an experimental preclinical study testing the applicability of autologous skeletal muscle-derived cells as a treatment of SUI in a canine model.

Methods:

Ten mongrel dogs were included in this study. Skeletal muscle was harvested for biopsy in 4 dogs. One month later, incontinence was induced in 8 dogs through urethrolysis. Biopsied muscle cells were incubated and expanded for 8 weeks. Muscle-derived cells were collected and covered with a polyglycolic acid (PGA) scaffold immersed in culture medium and coated with Matrigel to be used as a sling, which was placed suburethrally in 8 dogs; 4 had cell seeding, and 4 had scaffolds only. Urethral pressure (UP) measurements were performed at baseline and 2 & 6 weeks after sling insertion. The urethra was harvested 4 weeks after sling insertion for histopathology.

Results:

UP showed an increase in maximum urethral pressure during static measurement in all dogs with a scaffold inserted. The increase ranged from 5–40 cmH2O. Histopathology showed significant periurethral proliferation of skeletal muscles in 4 dogs with cell-seeded scaffolds. These levels were the maximum levels in dogs # 1 & 2. This was not the case in the 4 dogs that had slings only.

Conclusion:

The use of skeletal muscle-seeded PGA scaffolds is a practical technique with preserved histological differentiation integrity in a canine model.

Brief Summary

Autologous skeletal muscle-derived cells could be propagated in vitro, seeded into PGA scaffolds and used as slings.

Background

The treatment of stress incontinence is challenging [], and different surgical techniques have evolved throughout numerous clinical trials to efficiently treat this condition []. To date, no procedure is considered the “gold standard”. However, a midurethral sling (MUS) is the most widely used procedure and is considered the standard of care in women with stress incontinence []. Synthetic MUS have been used for decades but are not without risks and sometimes can be life threatening []. Many trials, both experimental and human, have attempted to provide an autologous, efficacious and durable tissue-engineered sling.

We conducted this experimental trial with the purpose of assessing the potential of autologous cell seeding of a biodegradable scaffold in treating stress-induced incontinence in canines.

This study evaluated a biodegradable *seeded (PGA) scaffold* enriched with autologous skeletal muscles without the need for a harvest procedure such as that used during the harvest of the rectus fascia sling [], which is the prototype for autologous slings [].

Materials And Methods

The study comprised 10 mongrel female dogs. Approval from the local ethics committee was obtained (Research Center Ethics Panel, Urology & Nephrology Center).

All methods were carried out in accordance with relevant regulations and in accordance with ARRIVE guidelines, including housing, anesthesia and surgical procedures in dogs.

Open episiotomy was carried out 2 weeks prior to the study to facilitate the vaginal approach.

Group 1 comprised 4 dogs in which a cell-seeded sling was applied, while in Group 2, only the PGA sling was applied.

Isolation and expansion of muscle-derived cells (MDCs):

In 4 dogs (Group 1), a muscle biopsy was harvested from the biceps under general anesthesia. The obtained biopsy averaged 0.2 g and was incubated in 0.2% collagenase type 1A in Dulbecco's modified Eagle's medium (DMEM) for 20 minutes at 37 °C (Sigma–Aldrich®, St. Louis, USA) digestion. Cells were cultured on laminin-coated tissue culture flasks in SKGM-2 medium (Lonza®, Valkersville, MD). The medium was replaced every 3 days. At confluence, cells were passaged and split 1:2 into tissue culture flasks. After 8 weeks and 4 passages, MDCs were collected for transplantation.

Characterization of MDCs:

The morphology of the cells was evaluated using phase-contrast microscopy. The expression of Desmin was assessed by immunohistochemistry (IHC) using monoclonal anti-Desmin antibodies (DakoCytomation®, Glostrub, Denmark).

Cell seeding on scaffolds:

A Neoveil absorbable PGA sheet (Gunze Limited®, Kyoto, Japan) was used in 2x3 cm segments. Seeding was started by immersion in culture medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin for 24 h at 37 °C before seeding. The scaffold was coated with Matrigel, and MDCs were seeded at a concentration of 1 million cells/cm^2. Matrigel was used in our study to increase cell adherence to the scaffold, as it is a promising material for this application [[i]]. After 24 h, the seeding side was flipped, and the other side was treated the same way. Both surfaces were fully

immersed in medium during the seeding process. The seeded scaffold was cultured for an additional 3 days in the same culture medium.

- **Induction of incontinence:**

One month after biopsy, incontinence was induced in 8 dogs through midline suprapubic incision and identification of the urethra and bladder. The urethra was sharply dissected all around the structure until disruption of the periurethral sphincter was achieved. This method is similar to that previously described by Rodriguez et al. [[ii]] with the addition of pubourethral ligament disruption.

- Two weeks after induction of incontinence, the sling was applied through a vaginal incision in the suburethral position in all 8 dogs: cell-seeded scaffold in the first 4 dogs and scaffold only in the other 4 dogs. The sling was fixed to the periurethral tissue. Urethral pressure (UP) measurements were performed before (baseline) and 2 weeks after insertion of the sling.
- The urethra with its surrounding tissue was harvested, and the dogs were sacrificed 4 weeks after sling insertion. Figure 1 shows the scaffold and steps of the surgical procedure.
- The urethra was fixed in 10% buffered formalin and processed in paraffin blocks, sectioned at 5 μm and stained with hematoxylin and eosin (H&E), methenamine silver (for reticuline fiber detection) and Masson trichrome stain (for fibrosis detection and muscle bundle delineation).
- Two dogs were considered controls, in whom no urethrolysis or insertion of slings was carried out. UP was carried out in these dogs before they were sacrificed, and their urethras were taken as controls.
- For IHC, Desmin antibodies were used (Abcam, Cambridge, UK) at a dilution of 1:100. Nerve fibers were identified through staining with polyclonal anti-S100 (DAKO, Carpinteria, CA) at a dilution of 1:200. Immunolabeling was performed using an avidin–biotin detection kit (Vectastain Elite ABC, Vector, Bulingame, CA). All sections were counterstained with Gill's hematoxylin.

A flowchart for the research procedures has been provided (Supplement).

Results

- UP shows an increase in maximum urethral pressure in all dogs with a scaffold inserted. Table 1 demonstrates the change in UP over time. The median maximum urethral closure pressure (MUCP) at baseline in the first group, where the scaffold was seeded with skeletal muscle cells, was 28.5 cmH2O, which increased to 67.5 at 2 weeks and 65.5 at 6 weeks. In Group 2 (scaffold-only group), the median MUCP increased from a basal value of 19 to 21 cmH2O and remained at this level at 6 weeks. The increase in the MUCP ranged from 13-60 cmH2O (median 39 cmH2O) in the first group. In the second group, the increase in MUCP ranged from -3 to 18 (median 5.5 cmH2O).

- Histopathology:

Luminal diameter, epithelial lining, lamina propria thickness with its elastic and collagenous fiber content (normally approximately 70% of whole sphincter thickness), fibrous tissue deposition, thick-walled vein condition (stratum cavernosum), and smooth and striated muscle (both constituting approximately 30% of total urethral thickness) [[i]] were assessed.

In Group 1, the urethral lumen was slightly dilated in comparison to the control group; the mucosal lining was of near normal thickness. The lamina propria was slightly thicker, and there was no evidence of fibrous tissue deposition, as evidenced with Masson trichrome staining. The reticular fibers were the same as those in the control group. Thick-walled veins were mildly increased in number and caliber compared to the control group. The overall muscle layer appears as lavender red bundles in Masson trichrome and is normal in thickness with some degrees of crowding and disorganization. Areas of newly formed muscle bundles with central nuclei were confirmed.

In Group 2, the damaged sphincter shows a mildly dilated luminal area, with an atrophic urothelial lining. The lamina propria thickness is increased with moderate fibrous tissue deposition in addition to lower reticular fiber content and mild inflammatory lymphocytic infiltrate. The vascular component is unremarkable. There is substantial loss of the muscle layer with an overall decreased wall thickness.

IHC for anti-desmin showed significantly higher sphincter muscle mass in seeded scaffold specimens than in the other group. Newly formed muscle bundles are wider and more randomly aligned than the normal sphincter. Staining for S100 shows the presence of nerve fibers between the regenerated sphincter muscles. Figures 2 and 3 show the histopathological changes in the two groups.

Discussion

Induction of incontinence by urethrolysis with pubourethral ligament injury was our approach of choice. It results in disruption of the native support of the urethra and durable loss of urethral resistance []. Incontinence was confirmed by a lower maximum urethral closure pressure compared to control dogs, in which no urethrolysis was carried out.

Myoblasts have been used as a treatment for stress incontinence in animal models by means of intraurethral injection []. In 2014, a similar approach was used in 35 adult women. Gras et al. [] described a technique performed largely under local anesthesia/intravenous analgesia. An open muscle biopsy was obtained from the vastus lateralis muscle and was “minced” and injected as a suspension in the same session via the periurethral route in 35 women under vaginal US. Cure/improvement was noted in 7–63% of the patients.

Other human trials involving intraurethral injection of muscle-derived stem cells have been published in both adult women [] and children []. Although the results were promising, some reports turned out to be unfounded [].

Another research strategy involved making structured skeletal muscle tissue in vitro, with the potential of making an all-autologous sling. Many studies have evolved to reconstruct skeletal muscle tissue. Some

focused on developing self-organizing tissues without artificial scaffolds []; others preferred seeding cells on natural or synthetic biodegradable substrates, e.g., collagen matrices []

We preferred polyglycolic acid as a biodegradable scaffold, as used by Saxena et al. [&], where myoblasts derived from neonatal rats, Fisher CDF-F344 cells, were seeded onto polyglycolic acid meshes and implanted into the omentum of syngeneic adult Fisher CDF-F344 rats with promising results and a comprehensive description of the approach. The advantage of the sling approach is that it applies a potentially successful technique [] with the added effect of the addition of autologous skeletal muscle fibers to the mid-urethra.

To our knowledge, no human study involving biodegradable slings seeded with autologous muscle-derived cells has yet been reported. Such a study will be of paramount importance, considering that the current treatment options for women with SUI are far from optimum []

The question we tried to answer is whether muscle-seeded biodegradable scaffolds are any different from plain scaffolds. Therefore, we compared absorbable slings to slings seeded with autologous muscle cells.

Cell-seeded slings proved to be more efficacious than plain PGA. Urethral pressure measurement 6 weeks after sling insertion showed that the median increase in urethral closure pressure in the cell-seeded sling was over 40 cm H2O, while in the other group where only PGA sling was applied, it was 5.5 cmH2O.

Histopathological study of harvested urethral segments 4 weeks after insertion of slings proved the persistence of viable skeletal muscle and nerve fibers.

This sling design avoids polypropylene-related adverse events. Application of this technique in humans is easy and promising based on functional and urodynamic outcomes.

One of the shortcomings of our study is reliance on the measurement of maximum urethral pressure in female dogs, which might not be very reproducible. This is probably true according to one study []; however, others [] have adopted the same parameter we used.

Conclusion

A biodegradable PGA sling seeded with autologous myoblasts showed evidence of survival 6 weeks after insertion in dogs. The use of skeletal muscle-seeded PGA scaffolds is a practical technique with preserved histological differentiation in a canine model in the short term.

Abbreviations

DMEM: Dulbecco's modified Eagle’s medium

SUI: stress urinary incontinence

IHC: immunohistochemistry

PGA: polyglycolic acid

MDCs: muscle-derived cells

UPP: urethral pressure profile

Declarations

Ethics approval:

The study was approved by the local ethics committee of the hospital.

Consent for publication: Not applicable

Availability of data and material:

The authors do not wish to share their data because of limited resources.

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Competing interests:

The authors declare that they have no competing interests

Author contributions:

All authors read and approved the final manuscript

Wadie, B S: Performance of the surgical procedures and urodynamics for all dogs. Shared in the design and drafting of the manuscript

Aamer, H G: Care for the animals during the period of the experiment and assisted in performing surgical procedures

Khater, S M: Histopathology

Gabr, M M : Preparation of the cell culture and propagation of skeletal muscle cells as well as seeding of PGA scaffolds

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Tables

Table 1: Maximum urethral closure pressure (MUCP) over time

Dog#	Baseline (cmH2O)	2- week post sling (cmH2O)	6-week post sling (cmH2O)
1	25	40	42
2	32	45	43
3	30	90	98
4	27	90	88
Median	**28.5**	**67.5**	**65.5**
5	20	38	37
6	21	18	20
7	17	20	19
8	18	22	22
Median	**19**	**21**	**21**
9	40	NA	
10	44	NA	

Figures

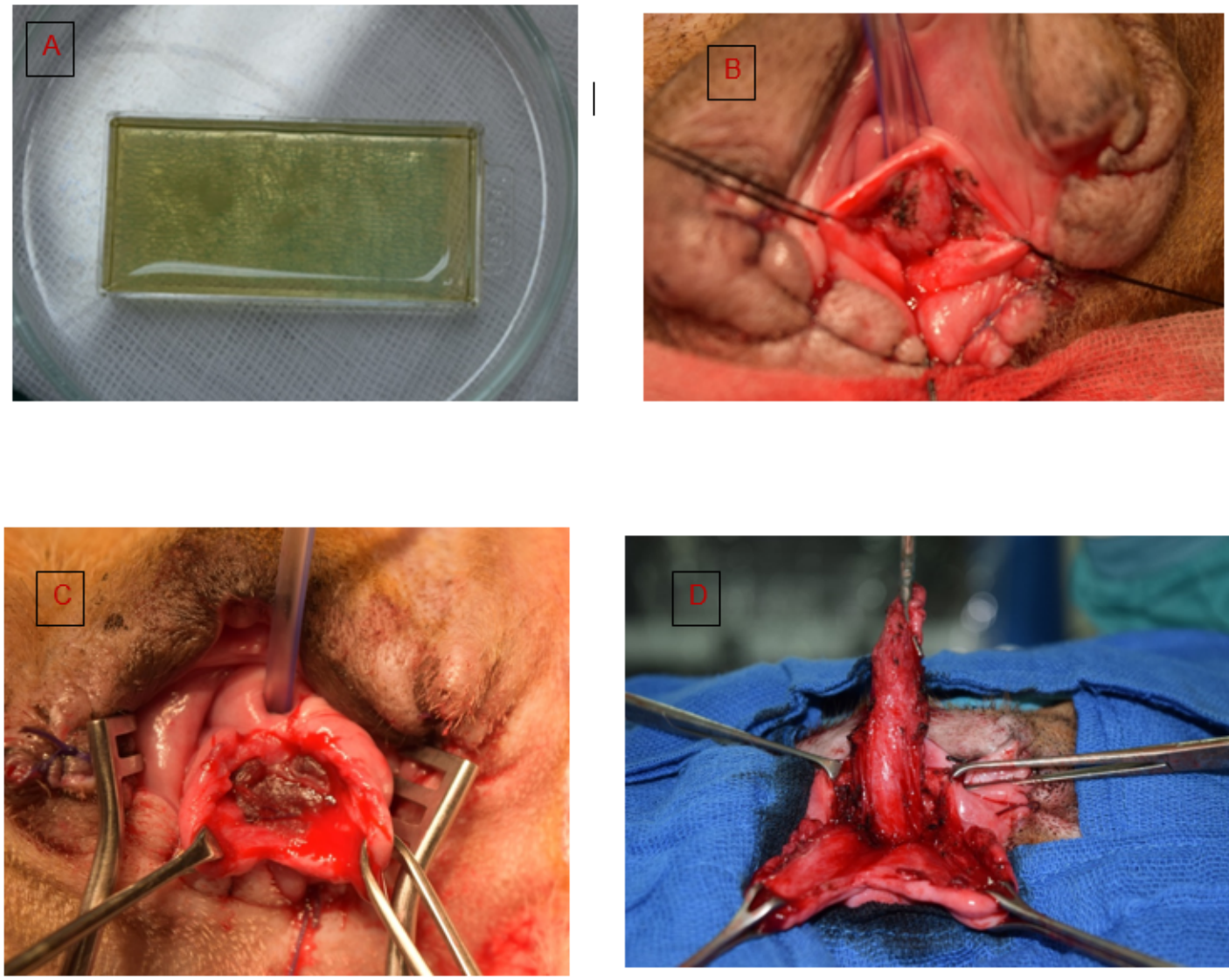

Figure 1

A) Agar plate with sterile medium and the scaffold immersed. The mesh of the scaffold is seen impregnated with seeded muscle cells, B) Dog urethra exposed and dissected clear C) PGA sling inserted sub-urethral and fixed by 5/0 stitches D) Whole urethra is being harvested after 4 weeks after insertion

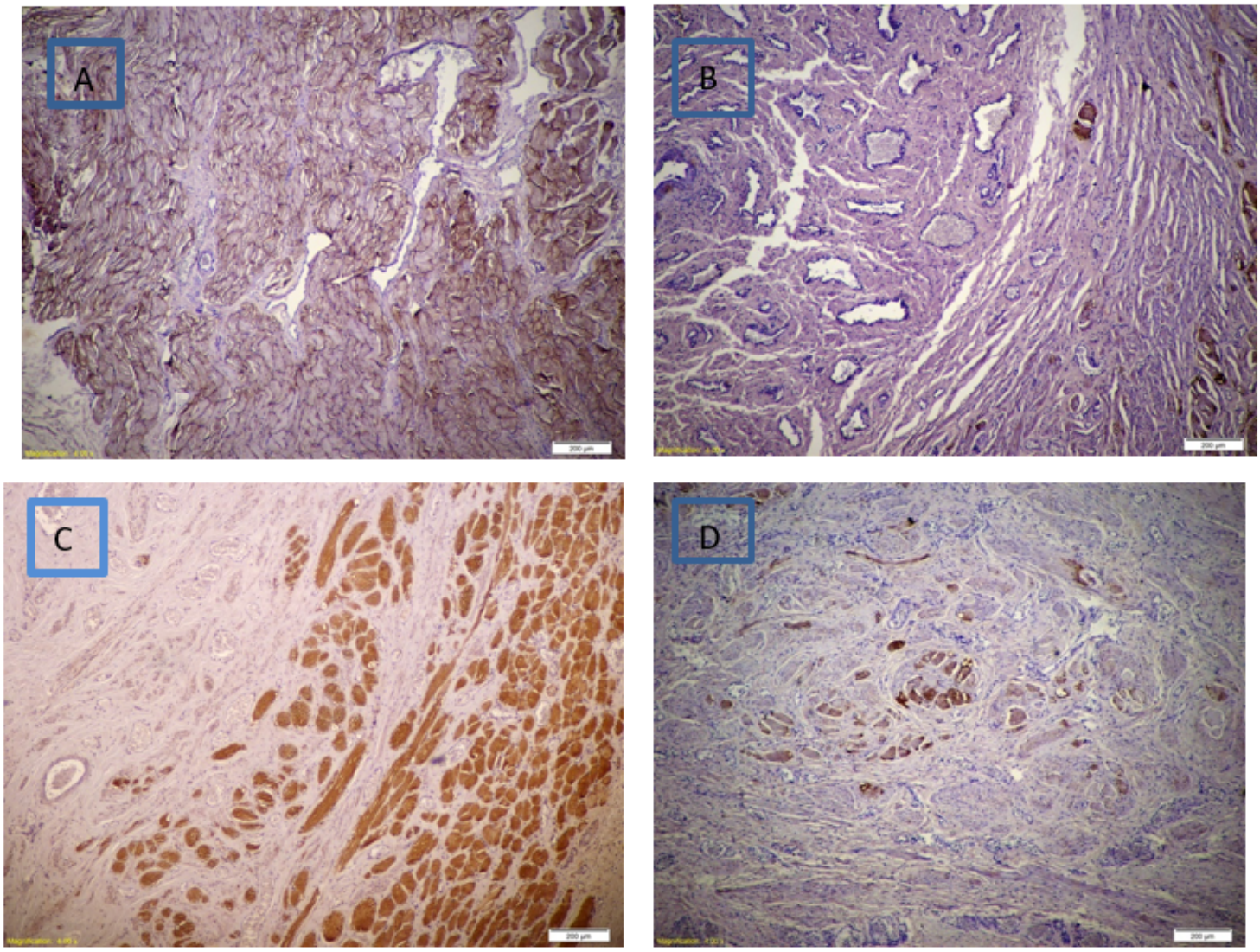

Figure 2

A} Diffuse increase in thickness of skeletal muscle bundles with mild disorganization in PGA seeded with MDC using IHC for desmin stain (brown), B} Minimal increase in thickness of skeletal muscle bundles in PGA only using IHC for desmin stain (brown), C} Abundant nerve fibers in between muscle bundles in PGA seeded with MDC using IHC for S100 stain (brown), D} Minimal increase in nerve fibers in between muscle bundles in PGA only using IHC for S100 stain (brown)

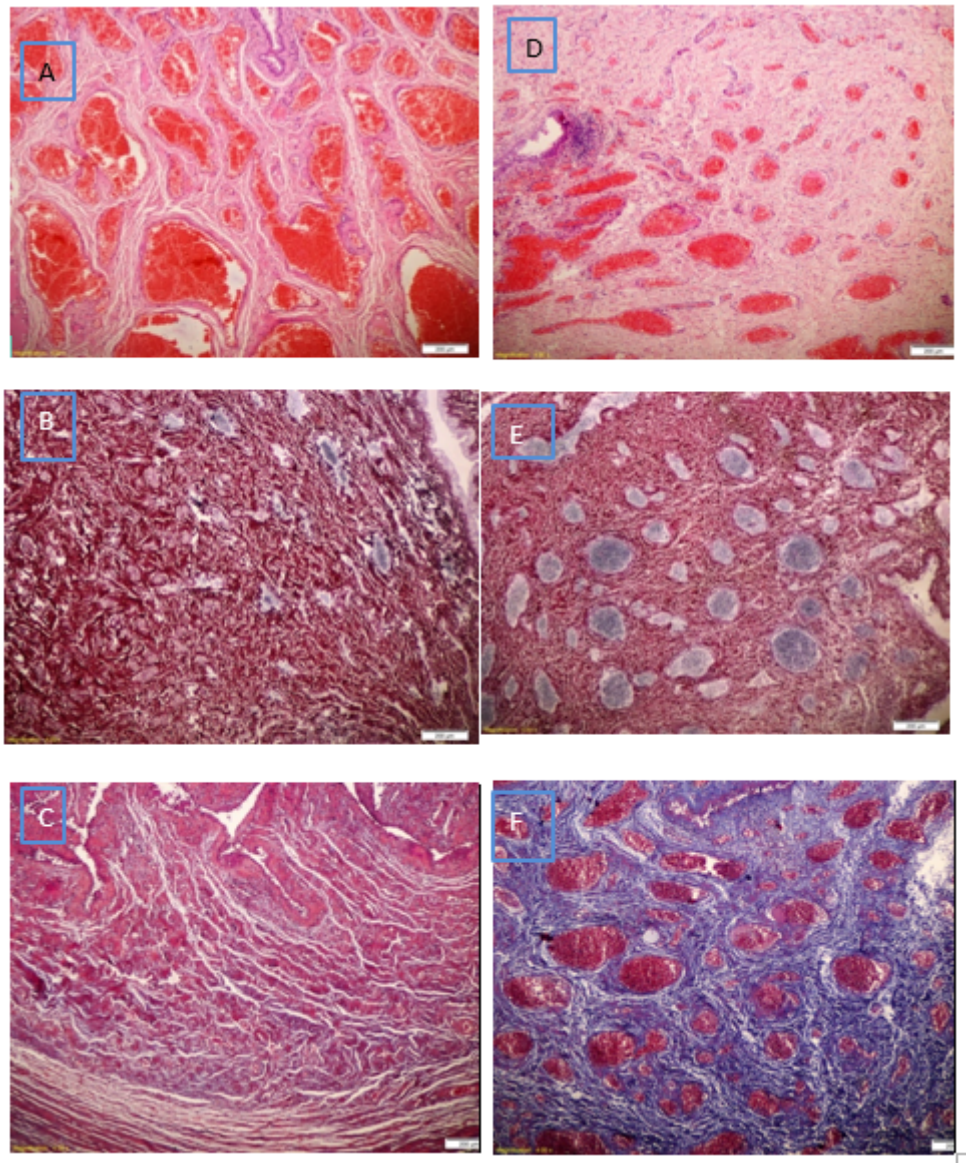

Figure 3

A} Venous plexus is increased in number with marked congestion and mild edema in PGA seeded with MDC, D} Venous plexus is near normal in number with mild congestion with mild inflammatory infiltrate in PGA only, B} Silver stain shows near normal amount of reticular fibers (black) in PGA seeded with MDC, E} Silver stain shows lower amount of reticular fibers (black) in PGA only, C} The lamina propria expressed absent fibrous tissue (blue) in PGA seeded with MDC using Masson trichrome stain, F} The lamina propria showed diffuse moderate fibrous tissue (blue) in PGA only using Masson trichrome stain

Supplementary Files

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- Flowchart.docx