APPLICATION OF HUMAN AMNIOTIC MEMBRANE (HAM) GRAFT IN THE DONKEYS PENILE CORPUS CAVERNOSUM DEFECT; DEVELOPING AN ANIMAL MODEL NEW METHOD FOR THE TREATMENT OF PEYRONIE’S DISEASE, PENILE CANCERS, AND CONGENITAL DEFORMITIES OF THE PENIS AND PENILE TRAUMA

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ABSTRACT

Purposes: To evaluate the efficacy of human amniotic membrane (AM) grafting in the Donkeys penile Corpus cavernosum defect; we developed an animal model new method for the treatment of Peyronie’s disease, penile cancers, and congenital deformities of the penis Materials and methods: 5 one-year-old donkeys of 150-200 kg weight has been included to our study. After induction of general anaesthesia penile skins were degloved and dorsal penile neurovascular bundles were dissected off the corpora cavernosa. Then a 4x5 cm rhomboid incision made at the mid part of the corpora cavernosa. Then a double folded patch of human amniotic membrane filled the induced defect after which the penile skin closure and sub cutaneous drain were made. After a two-month period all the animals were operated once again regarding penile deformity and stability, then total penectomy performed and the specimens sent to pathologist
for histopathologic exams. Using statistical and pathological assessments, all variables were evaluated. **Results:** Except in one animal which encountered severe penile infection and edema, all others animals well tolerated the recovery period. There were no death due the surgery, however in the first case there was a massive tissue fibrosis. Four healthy donkeys remained in our study. The average HAM patch size before the surgery was 4.65 cm (4.4 – 5 cm, SD= 0.3 cm) which became 4.20 cm (3.9-4.4 cm, SD= 0.22 cm) after an 8-week follow up surgery. The average patch contraction was 9.58% (4.5-12%, SD= 3.41%). No penile deformity or instability were seen in 4 animals. Histopathologic evaluation confirmed complete re-epithelialization of HAM. **Conclusions:** The amniotic membrane can be used as a suitable substitution for corpus cavernosum. It is safe, inexpensive, biodegradable, and available and may be used for the treatment of Peyronie’s disease, penile cancers, congenital penile deformities, and penile reconstructive surgery.

**KEYWORDS:** human amniotic membrane, HAM, corpus cavernosum, donkey.

**INTRODUCTION**

Human penile shaft is complex and composed of three separate cylinders. Tw paired cylinders called the corpus cavernosum make up the majority of the penis and the corpus spongiosum, as well as fascial layers, nerves, lymphatics, and blood vessels, all covered by skin. The two suspensory ligaments, composed of primarily elastic fibers, support the penis at its base. The penis of the horse is musculo-cavernous and can be divided into three parts: the root, the body or shaft, and the glans penis. The penis originates caudally at the root, which is fixed to the lateral aspects of the ischial arch by two crura (leg-like parts) that converge to form the shaft of the penis. The shaft constitutes the major portion of the penis and begins at the junction of the crura. It is attached caudally to the symphysis ischii of the pelvis by two short suspensory ligaments that merge with the origin of the gracilis muscles. The body is cylindrical but compressed laterally. When quiescent, the penis is soft, compressible, and about 50 cm long. Fifteen to 20 cm lie free in the prepuce. When maximally erect, the penis is up to three times longer than when it is in a quiescent state. Penile erection referred to a cascade of hemodynamic processes beginning with relaxation of smooth muscle of the corpus cavernosum and its associated arterioles. This relaxation process results in an increased blood flow into the trabecular spaces of the corpora cavernosa and eventually results in penile engorgement and finally to the penile erection. In 1962 Bourne described several layers in the amniotic membrane. The innermost layer, adjacent to the
amniotic fluid, is a single homogenous layer of cuboidal epithelial cells which is presumably derived from embryonic ectoderm. Certain characteristics of amniotic membrane make it a suitable substrate for surgery are Promotion of Epithelialization, Inhibition of Fibrosis, Inhibition of Inflammation and Angiogenesis, Lack of Immunogenicity, Antimicrobial and Antiviral Properties, High Hydraulic Conductivity.\[6-30\] Peyronie’s disease, penile cancers, congenital deformities of the penis, and penile reconstructive surgery are conditions in which a suitable substitute for corpus cavernosum is needed.

Despite several surgical methods and biomaterials, it seems that none of them is an excellent choice and more studies could be logical and the matter is still under discussion. We herein report our experience in excising a part of donkey’s penile corpus cavernosum, grafting the human amniotic membrane, and evaluating artificial erection and Histopathological changes of the specimens afterwards. We hope that success of this study could be as a new method for treatment of Peyronie’s disease and other conditions mentioned above.\[36\]

**MATERIAL AND METHODS**

The Human amniotic membrane (HAM) was separated from the remaining chorion by blunt dissection then it flattened on a clean paper with epithelium surface up. After blood clot cleaning with sterile saline solution it incubated in\[800000IU\] penicillin G. One week later, the paper adherent amniotic membrane cut in 3x4 width patches. We prepared HAM specimens brought out of the refrigerator just before transplantation. Just before the surgery a 4 cm length and 3 cm width HAM was prepared (39). Five healthy 1-year old male donkeys with an average weight of 150-200 kg were included our study. Fifteen minutes after induction of general anesthesia using intra-venous 0.6 mg/Kg acepromazine, an 18G angio-catheter was placed in the jugular vein and secured with two simple stiches to the neck skin. Twenty minutes after acepromazine injection, 1 mg/Kg xylazine was injected and after 5 minutes a cocktail of 2 mg/Kg ketamine- 0.05 mg/Kg diazepam was injected which induced general anesthesia. After endotracheal intubation the animal was put in the anesthetic machine. Then the animal was placed on the operating bed. The animal received IV antibiotic and anti-tetanus toxoid. In semi-dorsal position and after shaving and preparation of the surgical site with povidone-iodine solution, the rest of the body was covered with sterile drapes. Cefazoline 50 mg/kg and Gentamycin 3 mg/kg as prophylactic antibiotic was administered. Flaccid penis evaluated regarding any anatomical deformity. A tourniquet then was applied at the base of the penis. After inserting a scalp-vein catheter into the corporeal body, 200-250
mL of normal saline solution was injected until rigid erection happens and further injection was impossible. The penis was evaluated once again regarding any anatomical deformity. A long silicone catheter was placed. The penile skin degloved. Corpus spongiosum dissected of the corpus cavernosum. Dorsal neurovascular sheath and urethra dissected of the corpus cavernosum. Then a rhomboid incision 4x5 cm cm made at the mid part of the corporeal body in lateral aspect. Applying traction to the glans penis and opening this potential space, the double folded amniotic membrane graft with the same size as the defect was tightly sutured to its edges with prolene 5-0, the tourniquet was removed, and any bleeding from the graft edges was controlled by over-sewing with prolene 5-0. A perineal urethrostomy placed. A hemo-vac drain placed under the penile skin and penile skin sutured using 3-0 nylon stitches. The animal orchiectomized at the end of the operation to avoid unwanted erection. A pressure dressing was applied. All the animals became under 50 mg/Kg intera muscular cefazoline and analgesic therapy for 10 days.8 weeks after all the animals were operated once again and the gross anatomy was checked for any iatrogenic deformities in rigid and flaccid phases. Total penectomy with end urethrostomy performed and the sample was taken and was sent for histologic evaluation. A non-sacrificing technique performed for all of the animals and they left alive in the nature for the rest of their lives.

RESULTS

A HAM patch was covered the corporeal incision defect. The average HAM patch size before the surgery was 4.65 cm (4.4 – 5 cm, SD= 0.3 cm) which became 4.20 cm (3.9-4.4 cm, SD= 0.22 cm) after an 8-week follow up surgery. (Figure 1). The average patch contraction after 2 month was 9.58% (4.5-12%, SD= 3.41%). (Table 5)

<table>
<thead>
<tr>
<th>Patch size Changes</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<td>4.400</td>
<td>5.000</td>
<td>4.65000</td>
<td>.300000</td>
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<tr>
<td>Patch size After</td>
<td>4</td>
<td>3.900</td>
<td>4.400</td>
<td>4.20000</td>
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<td>Patch contraction (%)</td>
<td>4</td>
<td>.045</td>
<td>.120</td>
<td>.09581</td>
<td>.034198</td>
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There was not any case of major complication, mortality (0.00%), severe bleeding (0.00%), however, there was severe distal penile edema in the first case which was due to bad circumferential mucosal incision which lead to skin necrosis and severe inflammation of the corpora cavernosa and final massive corporeal fibrosis. (The first case was excluded because of technical error) Although there was severe tissue deformity in the first case, penile chordee
or deformity did not happened at the end of 8-week follow up in the rest of the cases. Microscopically examination of all 4 specimens reveals complete re-epithelialization of the surface epithelium. Sub epithelium reveals increased collagen fibers (confirmed by trichrome staining) method & decreased elastic fibers (revealed by elastin staining). One of the specimens (case 1) revealed ulceration of the surface epithelium with severe inflammatory changes without evidence of re-epithelialization and sub epithelial fibrin deposition and granulation tissue formation (figure 2)

Figure 1 - The appearance of penile tunica albuginea before grafting (A) 8 weeks after grafting (B) and severe edema and inflammation in case 1 (C).

Figure 2 - Histopathological examination of the normal corpus cavernosum (A, C, and E) and repaired area (B, D, and F). In slide A (H&E), the section is from the normal area and shows the squamous epithelium with papillary structures. In slide B (H&E), the section is from the grafted area after 8 weeks and illustrates complete squamous re-
epithelialization. However, the papillary structure of the epithelium became flattened to some extent. Also, scattered chronic inflammation was seen in this slide. In slide C, Masson’s trichrom staining of the grafted area was seen. In slide D, see severe edema and reactive inflammatory changes in case 1. Slides E and F demonstrate Verhoeff-van Gieson elastin staining of the normal and repaired areas, respectively. The comparison between these two slides shows decreased elastin fibers after grafting (Magnification: 100).

DISCUSSION

Peyronie’s disease is characterized by the development of a circumscribed, painless, dense, fibrous plaque toward which the erect penis angulates. In some patients the fibrous plaque is extensive and involves the whole circumference of the penis, and the septum results in penile shortening rather than angulation during erection. Definitive surgical management of Peyronie’s disease has long eluded urologists. For certain indications tunica preserving procedures have had reasonable success, the most popular of which are tunical plication and the Nesbit procedure. However, these procedures shorten the penis and do not correct the hourglass deformity. Graft replacement has gained wide popularity, and the use of many autologous and synthetic materials has been reported with varying results but notable shortcomings include contracture, curvature recurrence and impotence. Ideally a grafting procedure should result in a straight penis without impairing erectile function. Penile defects, which can be caused by iatrogenic penile injury or other reasons, are an uncommon, challenging condition that causes severe physiological and psychological problems. Loss of the glans, penile shaft and/or urethra, although rare, can occur as a complication of iatrogenic penile injury, burn or animal bite. The ultimate goal of reconstructive surgery is to have a penis with normal function and appearance. We have invent another surgical technique using human amniotic membrane for corpora cavernosal defects. Also to the best authors knowledge donkey’s penis carries the most similarities to the human’s penis regarding anatomic and physiologic features. As a matter of fact that we want to preserve the penile stability in rigid phase so we used patch graft in this study. The ability of HAM to be used in recovering defects in urology has been proven by Shakeri (urethra of rabbits), Ismail (ureter of dogs) and Salehipour (corpus cavernosum of dogs).

Shakeri used amniotic membrane to repair uroepithelium injuries in rabbits. All the samples revealed complete epithelialization of reconstructed urethra by non-keratinized
stratified squamous epithelium. The Histopathological changes showed a thin walled urethra with no definite muscle layer and absence of normal landmarks. There was only vascular proliferation and fibrosis suggestive of regeneration. Finally they concluded that amniotic membrane is an inexpensive, easy, and biodegradable graft with very little antigen effect which seems to be the ideal solution for urethroplasty.

Ismail and colleagues.\[35\] published an article to evaluate the feasibility of amniotic membrane as a graft in surgical reconstruction. Microscopically, there was apparent generous proliferation of the transitional epithelium, which was lining the amniotic graft, forming a complete tube. There was mild to moderate periureteral fibrosis in the previously mobilized parts of the ureters. However, there was no difference in the degree of fibrosis in the grafted site and the ureter above and below it. Examination of the grafted site revealed full thickness regeneration of the ureter. Finally they conclude that amniotic membrane has produced noteworthy results and its potential should be further evaluated in order to be used as a cheap, readily available source of graft for the various surgical reconstruction procedures. Park and colleagues.\[26\] designed a study to evaluate the clinical efficacy of amniotic membrane transplantation in the treatment of various ocular surface diseases. They finally concluded that, AMT is effective in promoting the re-epithelialization and suppressing inflammation, as well as producing complete remission of necrotizing scleritis and scleral ulcer with calcium plaque. The anti-inflammatory and anti-fibrotic properties of an AM have widened the therapeutic indications of AMT.

Salehipour and colleagues.\[36\] developed an animal model as the first step toward an innovating new method for the treatment of Peyronie’s disease, penile cancers, and congenital deformities of the penis. Histopathological evaluation revealed complete re-epithelialization of the epithelium with squamous layers. Finally they concluded that the amniotic membrane can be used as a suitable substitution for tunica albuginea. It is safe, inexpensive, biodegradable, and available and may be used for the treatment of Peyronie’s disease, penile cancers, congenital penile deformities, and penile reconstructive surgery. The results of the current study confirms previous studies and we observed a very nice a clean re-epithelialization of the HAM in corpora cavernosa of the donkey. However, as there is no physiologic erection in dogs and they have os penis, their study which showed no penile deformity and good anatomical and physiological function fails to resemble these good
results in human, however they showed that after 12 weeks there will be a very nice re-
epithelialization of HAM.

CONCLUSION
We conclude the feasibility HAM in repairing corporeal defects in donkeys which carries
minimal tissue deformity and irritation.

RECOMMENDATIONS
We highly recommend that this technique to be evaluated once again in donkeys and then
shifting this technique to be used in human volunteers.

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