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HISTOPATHOLOGICAL ASSESSMENT OF THE EFFECT OF NON-ABSORBABLE PROSTHETIC MESH ON THE SPERMATIC CORD CONTENTS: AN EXPERIMENTAL STUDY

By

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Background: ***Previous studies have documented that the use of synthetic prosthesis in repair of inguinal hernia markedly reduces the incidence of recurrence. However, little is known about the effect of these prostheses on the spermatic cord contents. This work was carried out to study the histopathological changes in response to contact with the commonly used prostheses (Prolene & Mersilene) on spermatic cord contents in male dogs.***

Materials and Methods: ***Thirty two male dogs were included in this study. The spermatic cords and testes were harvested from 2 dogs as a base line for the normal histology. The remaining 30 dogs were divided into 3 main groups. In the first (control) group (G1,n=10), the spermatic cord was delivered, its coverings were dissected and left intact on one side but on the other side it was skeletonized by removing its coverings. In the second group (GII,n=10), a strip of polypropylene (Prolene) mesh was wrapped snugly without strangulation around the dissected intact cord on one side and the skeletonized cord on the other side. A polyester (Mersilene) mesh was similarly applied in the third group (GIII,n=10). In each group, the spermatic cords and testes were examined histopathologically one week, and three months postoperatively.***

Results : ***At one week, the microscopic examination of the spermatic cord contents revealed more acute inflammatory reaction in mesh groups than the control. This reaction considerably subsided after three months with varying degrees and patterns of fibrous tissue deposition. These changes were more noticed in GIII than GII and in skeletonized cords than intact ones. The testes of all animals showed no histopathological changes.***

Conclusion: ***This experimental study proved the safety of the used non-absorbable prostheses on the spermatic cord contents and testes. Prolene mesh without skeletonization of the cord revealed more favorable results than Mersilene one .***

Key words: Mesh, Prolene , Mersilene , testis, spermatic cord, histopathology .

INTRODUCTION

Inguinal hernia repair continues to remain one of the most frequently performed surgical operation by the general surgeon[1,2] The use of prosthetic mesh repair proved to decrease the postoperative recurrence particularly in cases with weak posterior inguinal wall or recurrent hernias[3,4,5].

Several studies have demonstrated the histopathological changes of the anterior abdominal wall in response to different types of meshes after repair of an induced defect in experimental animals [6,7,8]. Recent studies reported an increase in the serum inflammatory response markers that indicates acute inflammatory process after inguinal mesh hernioplasty[9,10,11]. Furthermore, computed tomography (CT) and ultrasonography (US), revealing a thickened spermatic cord after implantation of meshes in the inguinal region[12]. On the other hand, some clinical study revealed no evidence of significant sexual function and testicular perfusion impairment after inguinal hernia repair with mesh[13]. However, little is known about the histopathological changes of the spermatic cord contents, namely the vas deferens, testicular vessels and lymphatics in response to synthetic meshes used in inguinal hernioplasty. So we **aimed** to study these changes with the commonly used prosthetic meshes (polypropylene "Prolene"& polyester "Mersilene") in male dogs as it is difficult and unethical to be evaluated in human beings.

MATERIALS AND METHODS

- ***Animal Groups*:**

This study included 32 adult male mongrel dogs, weighing 12-15 kg. Two of them were used as a base line

for the normal histology of the spermatic cord contents and testicles. The other 30 dogs were divided into 3 groups, each of 10 dogs.

The first group (GI,n=10) was the control group. In the second group (GII,n=10), the effect of polypropylene (Prolene, Ethicon Inc) mesh on the spermatic cord contents of intact and skeletonized sides were studied. In the animals of the third group (GIII,n=10), a polyester (Mersilene, Ethicon Inc) mesh was used instead of the Prolene one in the second group.

- ***Operative Procedures:***

All the operative procedures were carried out under general anaesthesia with endotracheal tube on the overnight fasting animals.

In each animal of the three groups, inguinal regions were sterilized on both sides with 10% providone-iodine (Betadine, The Nile Co. A.R.E) and draped. On each side, a four cm transverse inguinal incision starting 2 cm lateral to the midline was done. The spermatic cord was easily exposed and dissected from the surrounding tissues.

In the first group (GI), the spermatic cord coverings were dissected but left in place on one side (intact cord). On the other side, the coverings were dissected and excised to skeletonize the cord (skeletonized cord). The wound was closed with few interrupted subcutaneous stitches and continuous subcuticular 3/0 polyglactic acid (Vicryl, Ethicon LTD) suture.

In the second group (GII), a strip of Prolene mesh (2x4cm) was snugly wrapped without strangulation around the intact cord on one side and around the skeletonized cord on the other side. The 2 ends of the mesh strip were stitched to each other using interrupted 3/0 Prolene stitches.

In the third group (GIII), the procedure was the same as in GII but a Mersilene mesh was used instead of the Prolene one.

In only 2 dogs, the cord and testis were harvested to be the base line for their normal histology. They delivered through an inguinoscrotal incision. A high transfixion ligation of the spermatic cord was done using 0/Vicryl stitch below which the cord was resected with a scalpel. The cord in the specimen was divided distally just above the testis. The testis was preserved in picric acid (Bouin's solution) and the cord in 10% buffered formaline until the time of histological examination.

- ***Postoperative Follow up:***

The dogs were allowed water and milk for the first 24 hours postoperatively, and gradually for the normal diet afterwards. None of the animals included in this study received antibiotics.

The wounds and testes in all animals were clinically examined at one week. Five out of the 10 dogs in each of the 3 groups were explored one week postoperatively under general anaesthesia through an inguinoscrotal incision. The specimens of the testes and cords wrapped by meshes were recruited as mentioned above and preserved until the time of histological examination.

The cord and testis specimens of the remaining 5 dogs in each group were similarly harvested and preserved 3 months after the operation.

- ***Pathological Study:***

Gross examination of the spermatic cords, and testes of all animals was done.

Each mesh was examined in situ around the cord using a magnifying lens. It was unwrapped meticulously off the cord and the degree of fibrosis and adhesions to the cord tissues were evaluated.

Histological examination: Full thickness, cross sectional samples of the implanted meshes were examined regarding the pattern of fibrous tissue deposition. Representative testicular, epididymal and spermatic cord sections were prepared for histological examination. Spermatic cord cross sections were obtained around the site of mid-inguinal point in control group (GI) and at the site of mesh application in GII & GIII. Vasal luminal diameters were measured by identifying the vas in 90^{o} cross section and using a 10x ocular micrometer with a standard conversion factor.

Histological evaluation of the processed specimens were performed using hematoxylin and eosin (Hx& E) stain for inflammation and main pathologic criteria. Masson trichrome was used for better delineation of fibrous tissue. Sections were semiquantitatively assessed to determine the number of inflammatory cells present as well as the amount of collagen deposited. Further assessment of collagen was performed in 10 µm thick, sirius-red (Fluka chemica, Buchs, Switzerland)[14] stained serial sections. Morphometric quantitation of cord collagen deposition was done in 5 microscopic fields under a 20x magnification using a microscope (Zeiss, Axioskop, Germany) fitted with Kontorn Image Analysis System (software program, KS 400, Germany). Collagen content is represented as mean percentage of fibrotic area ± standard deviation (SD) of the total area measured microscopic fields in multiple sections of each subgroup. Data were analyzed in a standard form using the mean, standard deviation and analysis of varriance followed by LSD test[15]. A value was considered significant at $P < 0.05$.

RESULTS

In this study, the dogs withstood well the general anaesthesia and the surgical procedures. No single operative mortality was recorded.

- ***Clinical Examination:***

One week postoperatively, the wounds in the 30 studied animals were examined. Mild superficial wound infection was found in 3(30%), 2(20%) and 3(30%) dogs in GI, GII, and GIII respectively. These infected animals were left to complete the 3 months follow up. Only one case (10%) presented with a gaping wound with severe infection in the third group (Mersilene group). The cords and testes of the later animal were harvested and studied as it was difficult to manage the wound by frequent wash and dressing.

Four cases in GIII with skeletonized cords had clinical hydrocele. Two of them were explored at one week and the other two cases were left to complete the 3 months follow up.

Three months postoperatively, there was no noticeable wound infection in all animals although non of them received antibiotics. Also, no testes had hydrocele at that time.

- ***Histopathological Examination*:**

One week postoperatively; studied groups revealed swollen, pink, indurated spermatic cords in relation to the base line ones. These findings were evident in GII & GIII and more pronounced in the Mersilene group. In all groups, intact cords were more affected than the skeletonized ones due to involvement of their coverings.

The mesh was easily separable from the cord tissue especially in GII. There was no evidence of erosion due to mesh application.

Testes of the intact cords in all groups showed no abnormalities. In the skeletonized sides, subclinical hydroceles were discovered by gross examination in one case of GI and another one in GII. Also, the diagnosis of the two clinical hydroceles in GIII were confirmed.

Histologically, a foreign body reaction was noted in the mid portion of the spermatic cord of mesh groups in relation to control group (Figs. 1,2). There was uniform evidence of acute inflammation manifested by marked infiltration of neutrophil polymorphonuclear leukocytes and macrophages with skeletal muscle degeneration. The vessels showed marked congestion; the venules were affected more than the arterioles with focal lymphatic obstruction. All the cord specimens showed varying degrees of neural inflammation and early collagen deposition. The above changes were more pronounced in the skeletonized side of GIII (Table 1& 4; Figs. 3,4,5&6).

The wall of the vas was considerably thickened by marked edema, inflammation and early fibrous tissue deposition. Thickening of the vas deferens and decrease in cross sectional luminal diameter was observed in mesh groups particularly in the skeletonized side of Mersilene group, when compared to the base line control. The lumen was significantly decreased 14.82% and 19.06% by Prolene mesh in the intact and skeletonized sides respectively; 16.47% and 28.47% by Mersilene mesh compared to base line control (Table 2). Compared to the control group (GI), these changes were non significant in Prolene group while significant in Mersilene one ($P<0.05$).

Histological examination of all testes revealed normal spermatogenesis. There was no evidence of inflammation, fibrosis, or interstitial changes in the testicular parenchyma. The Leydig and Sertoli cell populations were indistinguishable between control and experimental mesh groups as was epididymal histology (Fig. 7).

Three months postoperatively; thickening and inflammation subsided and the cord became grayish white. The Mersilene meshes were more adherent to the spermatic cords when compared to the prolene ones.

No testicular abnormalities could be detected. The 2 clinical hydroceles previously diagnosed at one week completely disappeared.

Histologically, acute inflammation almost completely resolved. The spermatic cord tissues were infiltrated by chronic mononuclear inflammatory cells formed mainly of lymphocytes and histiocytes, with marked fibrous tissue reaction (Table 3& Figs. 8,9,10,11). The neural and vascular affection were less than that at one week. No focal changes could be identified in the nerves except for mild neuritis; however, the vascular channels showed mild thickening and congestion (Table 3& Fig. 12). The fibrous tissue formation was significantly higher in mesh groups (GII & GIII) than the control group, and more prominent in GIII (Table 4). The pattern of fibrous tissue formation varied in response to either types of meshes. In Prolene mesh, the collagen fibers formed moderate capsules concentrically oriented around each single mesh filament. Whereas, in the periphery, there was a thin scar plate oriented parallel to the mesh. In Mersilene prosthesis, the mesh was enclosed into a well-vascularized scar tissue, consisting of a frame of extensive fibrosis.

The wall of the vas relatively decreased in thickness with mild increase in the mean luminal diameter compared to those at one week (Table 2). The largest cross sectional vasal luminal diameter decreased 5.89% and 7.06% by Prolene mesh in the intact and skeletonized sides

respectively& 7.76% and 9.41% by Mersilene mesh compared to their base line and control groups. Both of these comparisons did not meet statistical significance (Table 2, Figs. 13, 14).

The testes and epididymes were quite normal in all animals.

Table (1): ***Histopathological changes of the spermatic cord contents one week postoperativly***

Reaction	*Groups*					
	Control (GI)		*Prolene (GII)*		*Mersilene (GIII)*	
	Intact	*Skeletonized*	*Intact*	*skeletonized*	*Intact*	Skeletonized
Acute inflammatory cells	+	++	++	+++	+++	++++
Tissue edema	-/+	+	++	+++	+++	++++
Granulation tissue formation	+	++	++	+++	+++	++++
Vascular thickening	+	++	++	+++	+++	++++
Neural inflammatin	-/+	+	-/+	++	+	++
Collagen tissue deposition*	_	_	_	+	+	++

*Collagen tissue deposition was assessed by Masson trichrome stain.
Reaction: (-) no, (-/+) weak, (+) mild, (++) moderate, (+++) marked, (++++) severe.

Table (2) ***: Changes in the mean vasal luminal diameter (Mean±SD).***

Postoperative timing	*Base line control*	*Groups*					
		Control (GI)		*Prolene (GII)*		*Mersilene (GIII)*	
		Intact	*Skeletonized*	*Intact*	*Skeletonized*	*Intact*	*Skeletonized*
One week	42.5±4.7	40±3.1	34.8±2.6**	36.2±2.9*	34.4±2.9**	35.5±3.4*	30.4±2.8***
Three months	42.5±4.7	42 ±4.1	41.3±4.2	40±4.3	39.5±4.1	39.2±3.9	38.5±3.8

SD: Standard deviation.
* Significant difference from base line control at P<0.05
** Highly significant difference from base line control at P<0.01
** * Highly significant difference from base line control at P<0.001

Table (3)*: Histopathological changes of the spermatic cord contents three months postoperatively*

Reaction	*Groups*					
	Control (GI)		*Prolene (GII)*		*Mersilene (GIII)*	
	intact	*skeletonized*	*Intact*	*skeletonized*	*Intact*	*Skeletonized*
Chronic inflammatory Cells	_	+	+	+	++	+++
Tissue edema	_	_	_	_	_	_
Granulation tissue formation	_	_	+	+	+	++
Vascular thickening	_	-/+	-/+	+	-/+	++
Neural inflammation	_	_	_	-/+	_	+
Collagen deposition	-	+	+	++	++	+++

Reaction: (-) no, (-/+) weak, (+) mild, (++) moderate, (+++) marked, (++++) severe.

Table (4): *Morphometric variation of collagen deposition within the spermatic cords in different groups using sirius red stain (Mean ± SD).*

Postoperative timing	Groups					
	Control		Prolene		Mersilene	
	Intact	Skeletonized	Intact	Skeletonized	Intact	Skeletonized
One week	14.68±2.16	15.04±2.13	16.46±2.64	19.89±4.01*	18.86±4.58	20.98±3.45**
Three months	16.56±2.12	17.96±2.11	18.16±3.39	24.06±4.88*	20.62±3.15*	30.64±4.17***

SD=Standard deviation
* Significant difference from the control group at P<0.05
** Highly significant difference from the control group at P<0.01
** * Highly significant difference from the control group at P<0.001

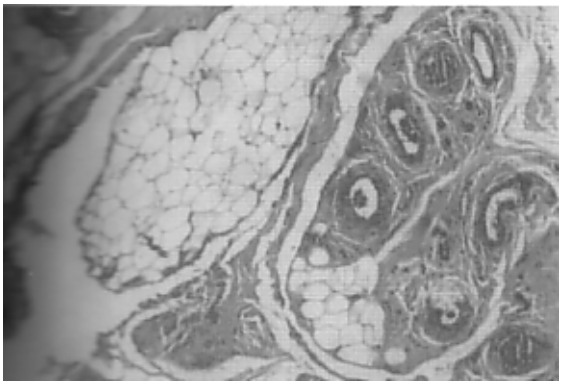

Fig.(1): *Histopathological findings of spermatic cord one week postoperatively in control group (GI) with intact cord (Hx.& E. x 200).*

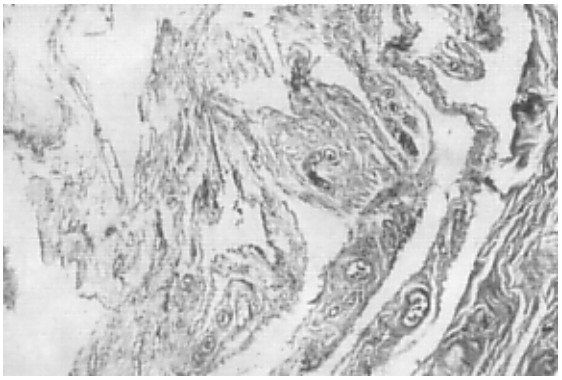

Fig.(2): *Histopathological findings of spermatic cord three months postoperatively in control group (GI) with intact cord (Masson trichrome x 200).*

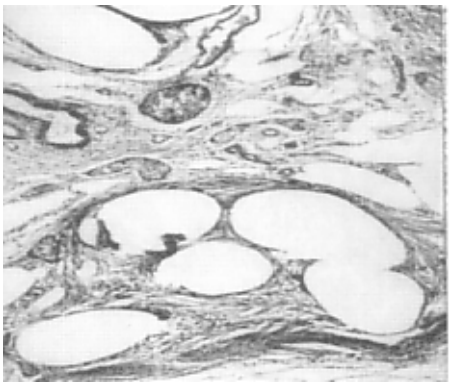

Fig.(3): *Histopathological findings of spermatic cord one week postoperatively in Prolene group (GII) with intact cord (Masson trichrome x 200).*

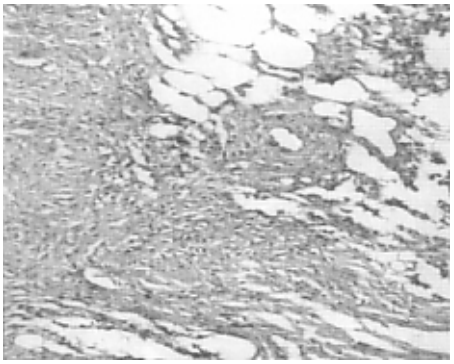

Fig.(4): *Histopathological findings of spermatic cord one week postoperatively in Prolene group (GII) with skeletonized cord (Hx.& E. x 200).*

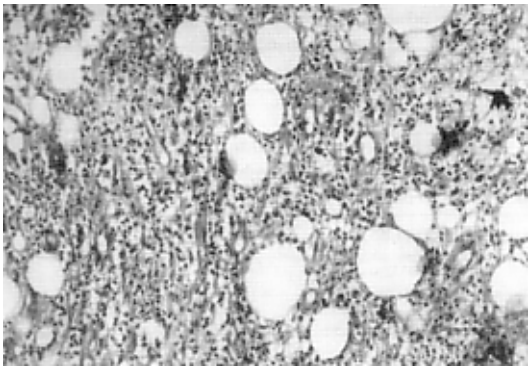

Fig.(5): ***Histopathological findings of spermatic cord one week postoperatively in Mersilene group (GIII) with intact cord (Hx.& E. x 200).***

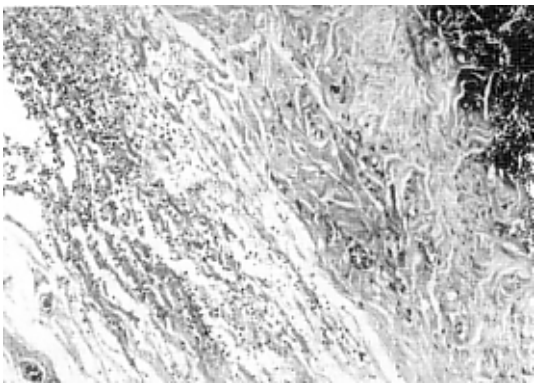

Fig.(6): ***Histopathological findings of spermatic cord one week postoperatively in Mersilene group (GIII) with skeletonized cord (Masson trichrome x 200).***

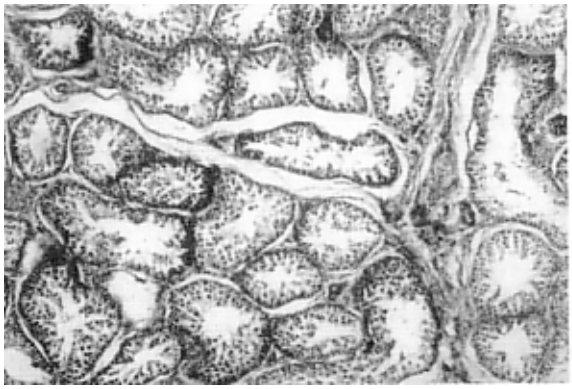

Fig.(7): ***Normal histopathological findings in the testis three months postoperatively (Hx.& E. x 200).***

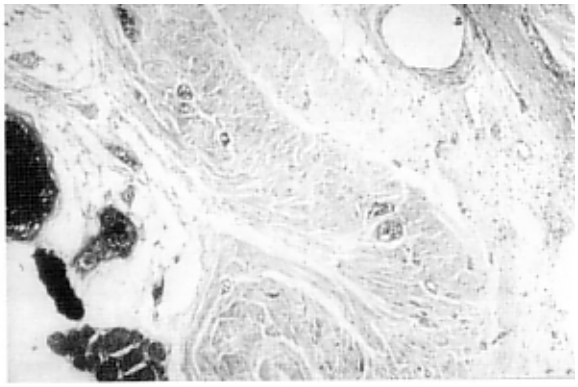

Fig.(8): *Histopathological findings of spermatic cord three months postoperatively in Prolene group (GII) with intact cord (Masson trichrome x 200).*

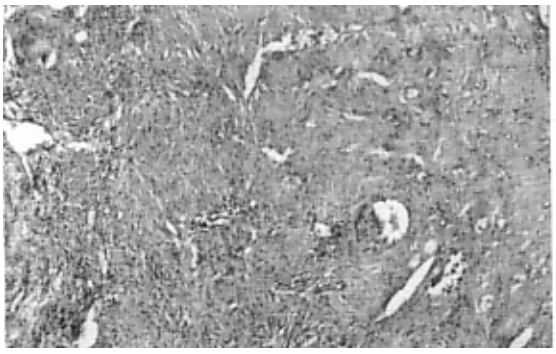

Fig.(9): ***Histopathological findings of spermatic cord three months postoperatively in Prolene group (GII) with skeletonized cord (Hx.& E. x 200).***

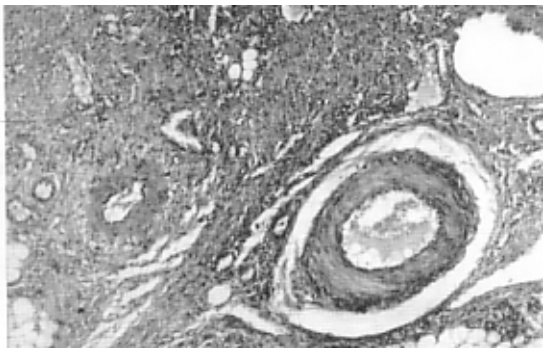

Fig.(10): *Histopathological findings of spermatic cord three months postoperatively in Mersilene group (GIII) with intact cord (Hx.& E. x 200).*

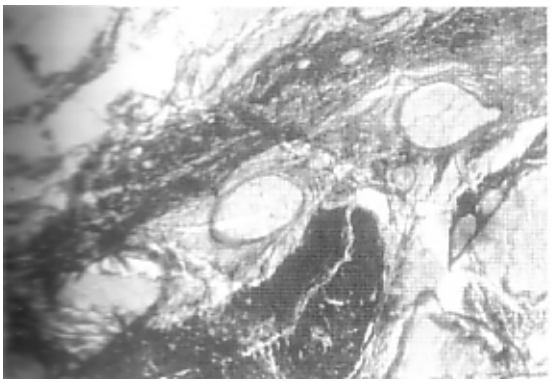

Fig.(11): *Histopathological findings of spermatic cord three months postoperatively in Mersilene group (GIII) skeletonized cord (Sirius red x 200).*

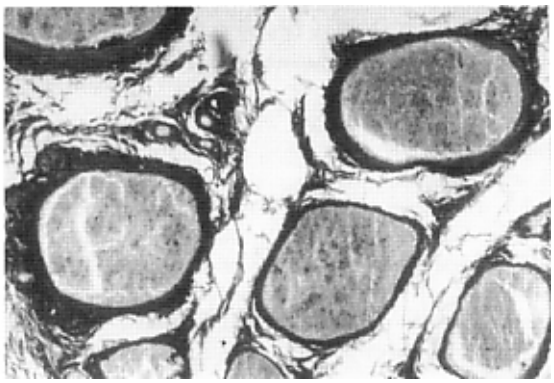

Fig.(12): ***Thickening and congestion of the vascular channels three months postoperatively (Sirius red x 200).***

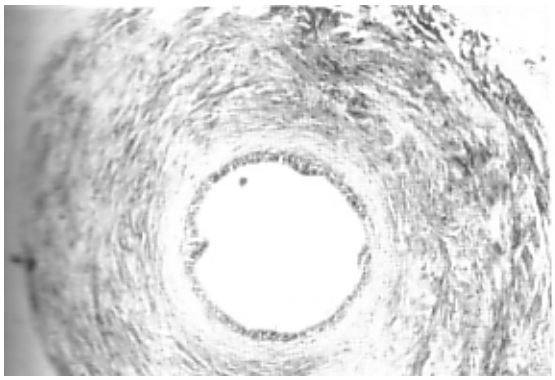

Fig.(13): ***Histopathological findings in the vas deferens three months postoperatively in Prolene group (GII) with intact cord (Masson trichrome x 200).***

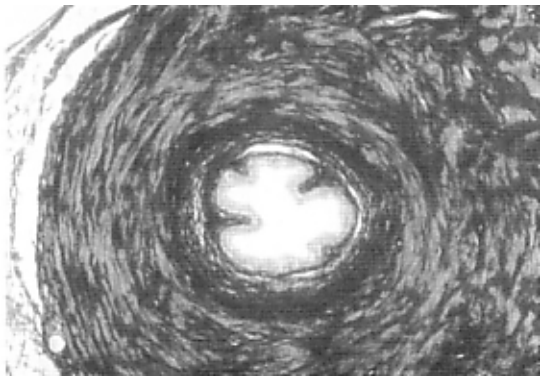

Fig.(14): ***Histopathological findings in the vas deferens three months postoperatively in Mersilene group (GIII) with skeletonized cord (Sirius red x 200).***

DISCUSSION

Non-absorbable monofilament polypropylene (Prolene) and multifilament polyester (Mersilene) meshes were used in this study as they are the most widely used materials in hernioplasty[16,17,18]. We did not attempt to simulate the technique of mesh repair of inguinal hernia in male patient for three reasons. The first of them, is that, dogs rarely develop inguinal hernia. Secondly, the aim of the present work was to test the effect of direct contact of mesh material on the spermatic cord contents and not to evaluate its efficiency to support the hernial repair which has been extensively studied in several previous clinical works. Lastly, if inguinal hernia is surgically induced in dogs and repaired using mesh, there might be no chance for all the spermatic cord contents to be in direct contact with the prosthetic material. So, we have preferred exposure of the cord contents to the tested prosthesis using the simple idea suggested and applied in this study.

In the meantime, previous studies reported a deleterious effect of the tight strangulating internal ring on the spermatic cord and testes. They reported cases of vascular thrombosis of the spermatic cord vessels, hydrocele, ischemia and atrophy of the testis [19,20]. So, in our study, the application of mesh was arranged to be non strangulating to obviate its mechanical constricting effect. Also, in clinical inguinal hernioplasty, some authors prefer to skeletonize the spermatic cord by removal of its coverings, leaving a thin cord to facilitate snug closure of internal ring without strangulation [21,22]. On the other hand, the majority do not recommend cord skeletonization as it

increase the incidence of vascular injury and testicular ischemia [23,24,25]. So, in this work, the effect of mesh was studied on the skeletonized and the intact cords in dogs.

The results of the present study revealed that monofilament polypropylene mesh did not increase the incidence of infection than that in control group. On the other hand, multifilament polyester mesh caused more incidence of infection than in the other 2 groups. These results agreed with those reported by other investigators who studied the effect of these types of meshes on the anterior abdominal wall in experimental animals. They proved a correlation between the mesh structure, surface and the size of their pores and the incidence of infection. They found that the monofilaments polypropylene (Prolene) mesh has no crevices and its surface is extremely smooth so that it is hardly colonizable by bacteria. On the contrary, the multifibered braided thread of polyester (Mersilene) mesh and its relatively rough surface make it less resistant to infection [12,22,26]. Also, the previous studies have documented that the biomaterial that contain pores of 10 μm in diameter or less may increase the risk of infection. This is because bacteria that are approximately 1μ (micron) in size can shelter in such spaces, where they are inaccessible to polymorphonuclear granulocytes which average 10-15 μm in diameter. Accordingly, the Prolene mesh of pore size 1.0-1.6 mm is more resistant to infection than Mersilene with a pore size of 0.6-1.0 mm [12,26,27].

In the current study, Mersilene mesh induced a more tissue reaction than prolene one. Although this may be considered as an advantage in ventral hernia as the subsequent fibrosis gives a more support to the abdominal wall [22,28,29], but theoretically, it might cause a potential damage or stenosis of the tubular structures of the spermatic cord, namely the blood vessels, lymphatics and vas deferens with possible fertility alteration.

In this study, the pattern of fibrous tissue formation varied in response to either types of meshes. In Prolene mesh, the collagen fibres were confined to each single mesh filaments. On the other hand, the Mersilene mesh was enclosed into a well-vascularized scar tissue, consisting of a frame of extensive fibrosis. These findings agreed with that previously reported regarding the anterior abdominal wall tissue response to these types of meshes [12]. Although there was no permanent damage to the tubular structures of the spermatic cord in our study, it might be suggested that this pattern of fibrosis makes them more likely to be at risk in Mersilene group.

It was also noticed that the vas deferens, blood vessels and lymphatics within the intact spermatic cord showed less inflammatory response than in the skeletonized one. It seemed that the spermatic cord coverings protect its contents not to be involved in the inflammatory process of the surgically dissected field or to come in direct contact with the prosthetic mesh.

In this experimental work, the inflammatory reaction of the spermatic cord contents considerably decreased 3 months postoperatively in all groups. Also, there was no significant decrease in the mean vasal lumenal diameter at that time. Furthermore, the testis in all animals included in this study showed no histopathological changes as a result of spermatic cord dissection or the contact with both types of meshes.

CONCULSION

The results of this experimental study proved that the used non-absorbable prostheses did not cause permanent adverse histological changes in the spermatic cord contents and testes in male dogs.

Regarding the safety of spermatic cord contents, Prolene mesh without skeletonization of the cord is suggested to be preferred to Mersilene one and excision of the cord coverings.

These experimental results might encourage the clinical use of Prolene mesh without skeletonization of the cord in inguinal hernioplasty.

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