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Damage to the spermatic cord by the Lichtenstein and TAPP procedures in a pig model

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Abstract
Background Mesh implantation is regarded as the standard treatment of inguinal hernias. Obstructive azoospermia induced by mesh implantation is a rare but serious complication. Whether different operative techniques or mesh materials used have an effect on the integrity of the testicle and spermatic cord remains unclear.
Materials In 12 minipigs a bilateral inguinal hernia repair, either open or laparoscopic, was performed using a standard small-pore polypropylene (PP) or large-pore polyvinyliden fluoride (PVDF) mesh. Next to measurement of the testicular size, thermography of the groin and testicle as a parameter for perfusion was performed preoperatively and at a follow-up at 6 months. Obstructions of the vas deferens were estimated radiographically. Testicular function (Johnson score) and mesh integration (granuloma size, apoptotic cells) were analyzed histologically.
Results Mean testicular size did not change significantly in follow-up compared to preoperative values. Technique and mesh material used failed to have a significant influence. Thermography of the groin following the Lichtenstein technique had significantly higher values at follow-up regardless of the mesh used. This could not been shown for laparoscopic treatment. Thermographic measurements at the testicle showed a significantly increased temperature in all groups compared to preoperative measurements. Only the Lichtenstein PP group showed significantly decreased values in testicular function. Quantity and quality of obstructions seen at vasography were most detectable in the Lichtenstein PP group. There was significantly decreased granuloma formation following PVDF mesh implantation compared to the PP mesh group regardless of the technique used.
Conclusions Both the technique and the mesh material have an impact on integrity of spermatic cord and testicular function. According to the results of this study, the laparoscopic TAPP procedure using a large-pore PVDF mesh has the least effect compared to preoperative values.

Keywords Hernia · Lichtenstein procedure · TAPP procedure · Tissue

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According to the European Hernia Society (EHS) guidelines, open or laparoscopic placement of a mesh prosthesis to patch the defect in the floor of the inguinal canal has to be regarded as the gold standard in groin hernia repair [1]. Fibroblastic ingrowth reinforces the abdominal wall and decreases the risk of recurrence. However, the implantation of alloplastic material is linked to foreign body reaction and chronic inflammation which are proportional to the weight, structure, and polymer of the mesh. Commonly used meshes contain too much material, producing an exaggerated foreign body reaction/tissue response leading to clinical complications [2–4]. To minimize the foreign body reaction and clinical complications, new types of mesh material have been introduced that have a decreased

amount of material and larger pores leading to nearby physiologic tissue ingrowth [3, 5, 6]. Further improvement of biocompatibility has been achieved using polyvinyliden fluoride (PVDF) as polymer [4]. PVDF is a polymer with improved textile and biological properties [7]. In comparison to polyester, PVDF is more resistant to hydrolysis and degradation. Furthermore, aging does not increase the stiffness of the mesh, which is seen with polypropylene. Although it has been used in vascular surgery for years, there are just limited types of surgical meshes until now. Whereas these so-called light-weight, large-pore, elastic mesh materials are known to have a favorable outcome with respect to postoperative pain [8–11] compared to conventional heavy-weight, small-pore, stiff mesh materials, just a few experimental studies focused on the effect of different mesh materials and different operation techniques on the integrity of the vas deferens. With widespread acceptance and ease of placement, the use of mesh in hernia repair is being offered increasingly to young patients whose fertility status may well be an issue in the future.

To further elucidate the impact of different mesh materials following Lichtenstein and TAPP hernia repair, an animal study using the pig was conducted to investigate the mesh materials' long-term effect on the integrity of vas deferens and testicular function.

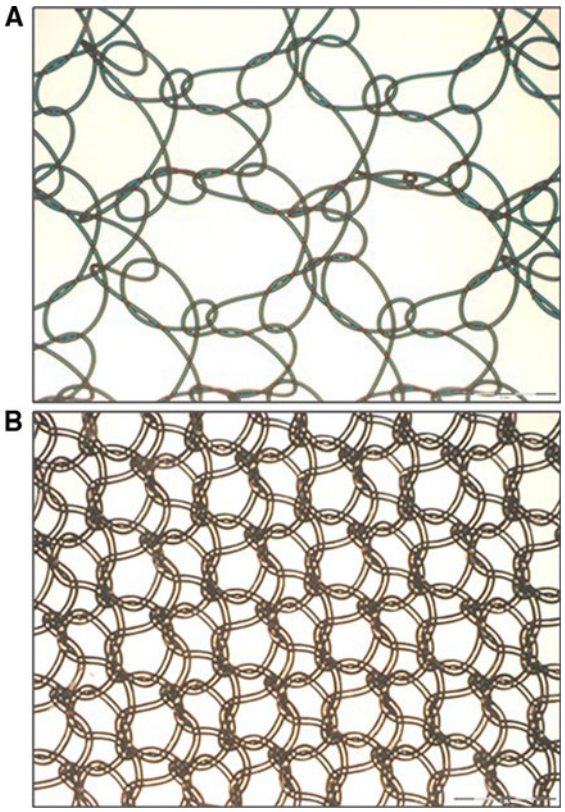

Fig. 1 **A** Large-pore and elastic mesh made of polyvinyliden fluoride monofilaments (PVDF). **B** A small-pore and stiff mesh made of polypropylene monofilaments (PP)

Materials and methods

The experiments were officially approved by the animal care and use review committee (TV AZ 9.93.2.10.35.07.087). All animals received humane care in accordance with the requirements of the German Tierschutzgesetz, §8 Abs. 1, and in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Washington, DC).

Mesh materials

Two different mesh materials were investigated: a large-pore and elastic mesh made of polyvinyliden fluoride (PVDF) composed in equal shares of a polymer blend of polyvinylidine fluoride and PVDF copolymer (95%) and 5% hexafluoride propylene and Marlex®, and a small-pore and stiff mesh made of polypropylene monofilaments (PP) (Fig. 1).

Animals

Twelve male uncastrated male pigs were housed under conditions of constant light and temperature and received a complete diet of feed and water *ad libitum* throughout the entire study, which was performed according to the NIH guidelines for the use of laboratory animals. All animals underwent bilateral Lichtenstein and bilateral TAPP hernia repair (one procedure on each side in each pig, $n = 12$ for each procedure).

Surgical procedure

Operations were carried out with the pigs under general anesthesia. Following premedication using 4 mg/kg Azaperon®, 44 µg/kg Atropin®, and 15 mg/kg ketamine i.m., an intravenous catheter was placed into an ear vein. Anesthesia was induced by injection of 10–15 mg pentobarbital. Anesthesia was continued by isofluran/oxygen as well as continuous infusion of fentanyl (45–90 µg/kg/h). Preoperative thermographic measurements were performed as a parameter of local perfusion using a VarioCAM® basic camera (InfraTec GmbH, Dresden, Germany) [12]. Following anesthesia induction, the skin was shaved and disinfected with povidone-iodine solution. An inguinal incision was made and the external oblique fascia dissected. Following exploration of the inguinal canal, a Lichtenstein procedure was carried out using a 7 × 10-cm slitted mesh sample with a small cutout for the spermatic cord (PVDF mesh on one side, PP mesh on the

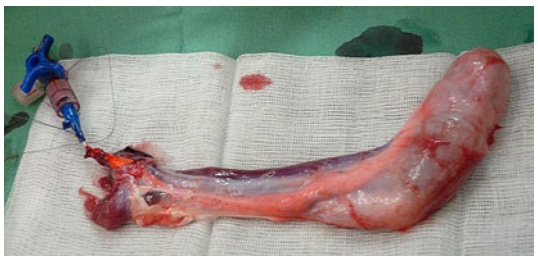

Fig. 2 Explanted inguinal area including testis with injected X-ray solution

contralateral side). Mesh samples were fixed at the inguinal ligament and the slit of the mesh samples was closed using 4/0 Prolene® sutures. Afterward, external oblique fascia and skin closure was performed. The TAPP procedure was performed following a small epigastric midline incision and insertion of a 10-mm trocar. Following CO_2 pneumoperitoneum (10 mmHg), two additional trocars were placed paramedian at the edge of the rectus muscle. A peritoneal incision was made at the level of the arcuate line. Blunt preperitoneal dissection allowed placement of a nonslitted 7 × 10-cm mesh covering all areas of potential herniation (PVDF mesh on one side, PP mesh on the contralateral side). Mesh was fixed medially with two to three nonabsorbable tackers. Peritoneal and fascial closure terminated the procedure. No antibiotic treatment was given before or during the experiments.

Throughout the whole observation period, all animals were objectively controlled and underwent daily clinical investigation to assess local and systemic complications. Six months after mesh implantation, all animals ($n = 12$) underwent thermographic investigation of the inguinal region as well as the testis. Thermographic measurements were performed as a parameter of local perfusion using a VarioCAM® basic camera. Following measurement of the size of the testicle by ultrasound, animals were sacrificed for morphological observations. The abdomen was opened for complete exploration. The intra-abdominal part of the vas deferens was dissected 2 cm before entering the inguinal canal at both sides and 10 ml of X-ray solution was injected for vasography (13.3 g gelatin, 16.6 g Bleimennige = Pb_2PbO_4, 100 ml water). Following ligation of the vas deferens, the whole inguinal canal, including the mesh samples as well as testis, was excised and immediately fixed in 10% formaldehyde (Fig. 2).

Assessment of integrity of vas deferens

The integrity of the vas deferens was assessed semiquantitatively using X-ray vasography. Obstructions of the vas deferens were classified as minor (0–25% reduction of lumen diameter), medium (25–75%), or major (>75%) and examined at the margins of the mesh samples as well as within the mesh area.

Table 1 Johnson score [13]

10	Complete spermatogenesis with many spermatozoa
9	Many spermatozoa present but germinal epithelium disorganized with marked sloughing or obliteration of lumen
8	Only few spermatozoa (<5–10) present in section
7	No spermatozoa but many spermatids present
6	No spermatozoa and only few spermatids (<5–10) present
5	No spermatozoa, no spermatids, but several or many spermatocytes present
4	Only few spermatocytes (<5) and no spermatids or spermatozoa present
3	Spermatogonia are the only germ cells present
2	No germ cells but Sertoli cells are present
1	No cells in tubular section

Histological analysis

Tissue specimens were embedded in paraffin. Histological investigation was performed on 3-µm sections after hematoxylin and eosin staining (H&E). All sections were processed at the same time to reduce internal staining variations. Spermatogenesis as the main testicular function was estimated histologically using the Johnson score [13]. The quantity of perifilamentary foreign body reaction was measured by analyzing the size of granuloma (in µm). TUNEL histochemistry for the detection of apoptotic cells was performed by an in situ apoptosis detection kit (APOPTAG, ONCOR, Cat. No. S7100, Germany). Sections were examined by standard light microscopy (Olympus BX51, Hamburg, Germany). For each sample five regions within the interface (400×, area = 100 µm × 100 µm) were captured by a digital camera (Olympus C-3030). The expression of immunohistochemical parameters was classified by two independent, blinded observers. Extent of staining was graded as the percentage of positively stained cells in the specimen (0–100%) (Table 1).

Statistical analysis

Statistical analysis was carried out using SPSS v17.0 (SPSS, Inc., Chicago, IL, USA). Data were organized according to the groups. Analysis of the Johnson score, of assessment of the integrity of the vas deferens, and of histology was performed using the Mann–Whitney U test. P values of less than 0.05 were considered to be significant. All data are presented as mean ± standard deviation if not otherwise mentioned.

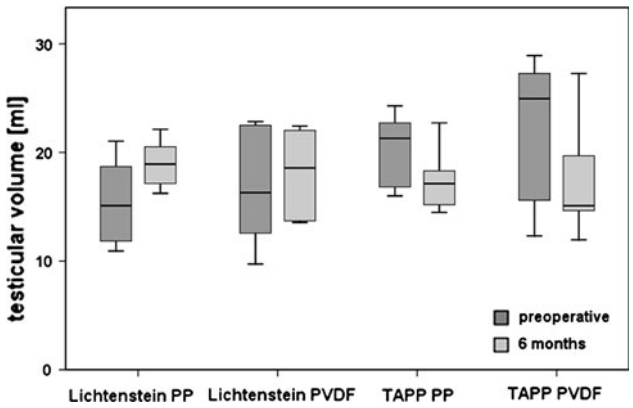

Fig. 3 Testicular volume preoperative and after a follow-up of 6 months

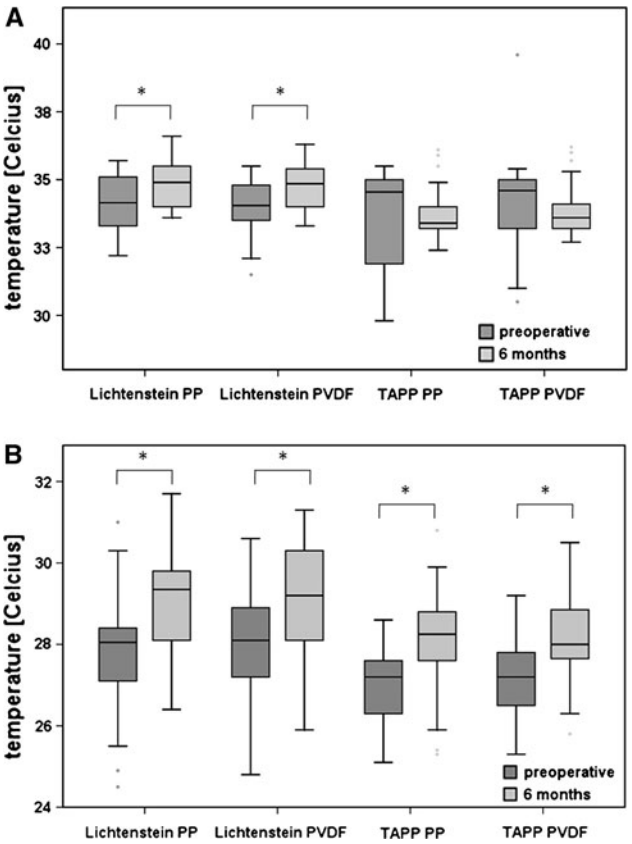

Fig. 4 Temperature at the **A** groin and **B** testicle preoperatively and after 6-month follow-up. Significant differences (*asterisks*) marked

Results

Macroscopic observation

The surgical procedure was well tolerated by all animals and the postoperative period was uneventful. None of the animals developed signs of ischemic orchitis or testicular atrophy. The mean volume of the testicles was 18.7 ± 5.5 ml preoperatively and 18.0 ± 3.7 ml after a follow-up of 6 months. There were no significant differences between the preoperative and postoperative values in all investigated groups (Fig. 3).

Thermography

Investigating the mean inguinal temperature at the groin, we found a significantly increased temperature in follow-up after the Lichtenstein repair, regardless the type of mesh used (Lichtenstein PP, 34.1 ± 1.0°C vs. 34.9 ± 0.9°C, $p < 0.01$; Lichtenstein PVDF, 34.1 ± 1.0°C vs. 34.7 ± 0.9°C, $p = 0.015$), whereas no significant differences were observed after TAPP repair. Comparing both procedures (Lichtenstein vs. TAPP), irrespective of the mesh material used, the groin temperature was significantly higher in the Lichtenstein group at follow-up ($p < 0.01$, Fig. 4A). The testicular temperature was found to be significantly higher at follow-up irrespective of mesh and method used (Lichtenstein PP, 27.8 ± 1.5°C vs. 29.0 ± 1.5°C, $p < 0.01$; Lichtenstein PVDF, 27.9 ± 1.3°C vs. 29.1 ± 1.4°C, $p < 0.01$; TAPP PP, 27.0 ± 0.9°C vs. 28.0 ± 1.2°C, $p < 0.01$; TAPP PVDF, 27.1 ± 1.0°C vs. 28.1 ± 1.0°C, $p < 0.01$). Again, comparing both procedures (Lichtenstein vs. TAPP), irrespective of mesh material used, the testicular temperature was significantly higher in the Lichtenstein group at follow-up ($p < 0.01$, Fig. 4B).

Integrity of vas deferens

Following explantation, X-ray vasography showed analyzable results in all mesh implantations. Overall, relevant obstructions (>75% of lumen diameter, Fig. 5) were located only at the mesh margins. Following Lichtenstein repair, four PVDF explants showed none or minor obstructions, and just one of six PVDF explants had an obstruction of 25–75% of the lumen diameter. The PP mesh group explants were found to have significant obstructions of more than 75% of the lumen diameter in two specimens as well as obstructions of 25–75% of the lumen diameter in another two. After TAPP repair, just two samples of the PP mesh group showed obstructions of 25–75% of the lumen diameter. Histologically, no direct infiltration of the mesh fibers into the vas deferens was found. Obstruction was more or less due to compression of the wall of the vas deferens, inducing an inflammatory and fibroblastic reaction in the wall of the vas.

Histological analysis

Testicular function was estimated histologically. Each testicular sample (10 tubuli seminiferi) was classified

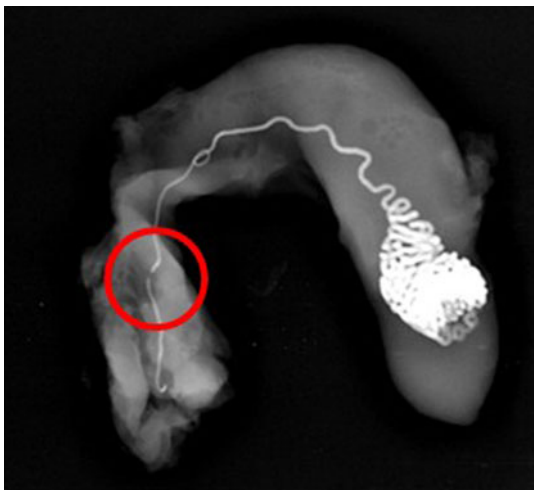

Fig. 5 Example of vasography. Relevant obstruction of the vas deferens at the margin following Lichtenstein PP mesh implantation

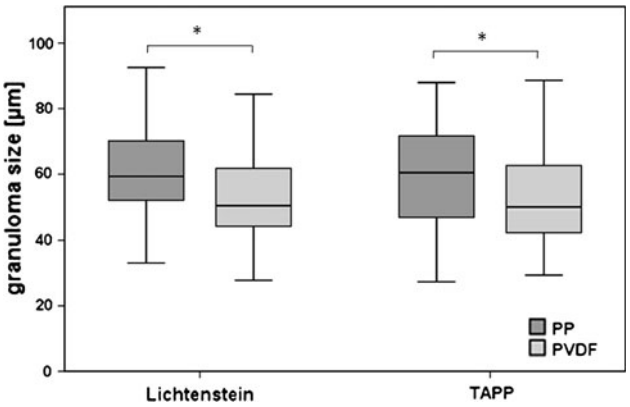

Fig. 6 Granuloma size (µm) at the interface of mesh/host tissue. Significant differences (*asterisks*) marked

according to the Johnson score. Compared to the preoperative values, just the Lichtenstein PP group was found to have a significant reduction (8.8 ± 0.1 vs. 8.4 ± 0.3, $p = 0.015$). No significant differences were found within all other groups (Lichtenstein PVDF, 8.7 ± 0.2 vs. 8.7 ± 0.2, $p = 0.43$; TAPP PP, 8.5 ± 0.4 vs. 8.5 ± 0.1, $p = 0.39$; TAPP PVDF, 8.4 ± 0.8 vs. 8.5 ± 0.3, $p = 0.93$).

Microscopic investigation of the mesh–host tissue interface showed typical formation of foreign body granuloma. The extent of foreign body formation measured showed significantly decreased granuloma following PVDF mesh implantation compared to the PP mesh group (Lichtenstein PVDF, 52.5 ± 11.9 µm vs. Lichtenstein PP, 60.7 ± 13.5 µm, $p < 0.01$; TAPP PVDF, 53.1 ± 14.0 µm vs. TAPP PP, 59.3 ± 14.8 µm, $p < 0.01$, Fig. 6). Significant differences were found in the percentage of apoptotic (TUNEL) cells just following TAPP repair, comparing the PP

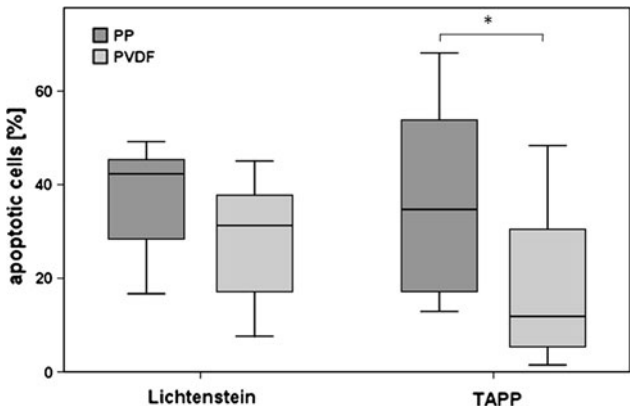

Fig. 7 Percentage of apoptotic cells at the interface of mesh/host tissue. Significant differences (*asterisks*) marked

(36.9 ± 19.3%) and the PVDF mesh samples (18.5 ± 16.4%, $p < 0.01$, Fig. 7).

Discussion

Obstructive azoospermia is a rare but serious complication following inguinal hernia repair. Whereas an incidence of iatrogenic perioperative injury to the vas deferens during inguinal hernia repair of 0.3% in adults and 0.8–2.0% in childhood has been described [14], little is known concerning the long-term effect of different mesh prostheses and operation techniques on the integrity of the vas deferens. Because almost 30% of patients undergo bilateral hernia repair and mesh repair is being offered increasingly to young patients whose fertility status may well be an issue in the future, this incidence rate is of major clinical importance. Next to case reports [15], just one larger clinical series on this subject has been reported. Shin et al. [16] investigated 14 cases of azoospermia secondary to inguinal vasal obstruction related to previous mesh herniorrhaphy. Following open or laparoscopic hernia mesh repair, they reported on nine patients with bilateral and five patients with unilateral obstruction. Surgical exploration revealed a dense fibroblastic response encompassing the polypropylene mesh with either trapped or obliterated vas in all patients.

Uzoo et al. [17] performed the first experimental study investigating this matter by comparing six dogs operated on with a polypropylene mesh to six dogs operated on with conventional (Shouldice) repair. Within their study a decrease in the cross-sectional diameter of the vas deferens on the operated side compared to the control side in both the suture repair and the mesh groups was found. Goldenberg et al. [18] investigated 18 dogs with a follow-up of 60 days and found a reduction of spermatogenesis as well as a reduction in the diameter of the lumen of the vas

deferens at the mesh side. Comparing open and laparoscopic mesh techniques in a clinical trial, Akbulut et al. [19] found testicular volume and testosterone levels to be significantly decreased after TEP compared with Lichtenstein, while no significant preoperative changes were observed between those groups. In contrast, we could not find significant differences in the testicular volume regardless of the technique and mesh used. Ersin et al. [20] investigated postoperative changes of blood flow following Lichtenstein and TEP and found significantly altered flow parameters for each group compared with preoperative, very early postoperative, and early postoperative values.

Concordantly, in our study the analyzed temperature (as a parameter of perfusion) showed significantly changed values at the groin following Lichtenstein repair and at the testicle regardless of operation and mesh used. The Lichtenstein repair induced a higher perfusion at the groin even 6 months after operation, probably because the mesh is located directly in the inguinal canal.

Studies comparing the effect of different mesh prostheses are rather limited. Peiper et al. [21] investigated spermatic cord perfusion and spermatogenesis in rabbits, comparing Lichtenstein hernia repair using UltraPro®, a low-weight large-pore, elastic mesh, and Marlex®, a heavy-weight, small-pore, stiff mesh of polypropylene, and the Shouldice repair. Using an IC-View system after removal of skin and subcutaneous tissue, they found a more obvious decrease of pure spermatic cord arterial perfusion after Marlex mesh repair than after Shouldice repair. Evaluating spermatogenesis, the Peiper et al. study showed a certain decrease in the Johnson score in seminiferous tubules after Lichtenstein repair independent of the kind of mesh. In contrast, in the present study, following Lichtenstein PP mesh implantation a significantly reduced Johnson score was estimated. In contrast, TAPP repair and the use of PVDF mesh did not alter the score. These findings again are consistent with our previous study [22] in which a significantly decreased Johnson score after the Lichtenstein using a stiff small-pore PP mesh compared to an elastic large-pore mesh was detected.

Berndsen et al. [23] compared a low-weight composite mesh (Vypro II) and a heavy-weight (Prolene) mesh used for Lichtenstein repair in rats. Ninety days after implantation, a median cross-sectional area of the vas deferens was 109 pixels at the Prolene and 158 pixels at the Vypro II mesh side without significant difference. Within our study, obstructions were analyzed semiquantitatively using vasography. Investigations revealed obstructions that were located mainly at the mesh margins. The stiff PP mesh group showed an overall higher amount and degree of stenosis compared to the PVDF mesh. Overall quantity and quality of obstructions were reduced following TAPP repair compared to Lichtenstein repair. The analyzed amount of obstructions measured following Lichtenstein repair agrees with a previous study of a rabbit model [22]. Within this study, vasography revealed relevant obstructions which were located at the mesh margins in up to 50% of the PP mesh samples (Lichtenstein). Besides significantly reduced inflammatory foreign body reaction compared to the PP mesh, the lower number of obstructions is probably due to the elastic textile properties of the PVDF mesh. In contrast to the study by Shin et al. [16], we could not find direct infiltration of the mesh fibers into the vas deferens. Obstructions were due mainly to compression of the wall at the mesh side with an induced inflammatory and fibrotic reaction in the wall of the vas deferens.

To summarize, great effort has been put into the challenge of creating a mesh material that optimizes a patient's outcome. The introduction of improved mesh materials has led to a superior outcome with respect to postoperative pain and foreign body feeling in both the early postoperative period and the long-term course. However, the effect of different mesh materials and implantation techniques on spermatic cord structures has not been studied thoroughly. For the first time a large-pore and elastic monofilamentous PVDF mesh used preferably in the TAPP technique showed a beneficial effect on the integrity of the vas deferens in this experimental setting.

Disclosures Drs. Junge, Klinge and Schumpelick have participated at courses and workshops regarding hernia repair organized by Ethicon, Norderstedt and Braun, Melsungen. Drs. Binnebösel, Kauffmann, Rosch, Klink, von Trotha, and Schoth have no conflicts of interest or financial ties to disclose related to this article.

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