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Morphological Alterations of Rabbit Oviducts Following Ligature of the Tubal Isthmus

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Summary. An animal model of tubal sterilisation would be valuable in order to examine the problem of severe damage of the tubal wall and to study the criteria for optimal anastomosis.

In a preliminary experimental group the Fallopian tubes of 30 rabbits were ligated at the isthmic segment. Specimens of the ligated portion and other tubal segments have been investigated by light microscopy and scanning and transmission electronmicroscopy one, four and twelve weeks following the ligature.

Changes in the Fallopian tubes following the ligature were limited to the ligated portion. The morphological findings consisted of a flattening and decrease of differentiation of the mucous epithelium, and of a slight scarring in the tubal wall and in the subperitoneal tissue within the constricted segment.

Tubal ligation at the isthmic segment provides good conditions for refertilisation, following resection of the altered portion and microsurgical anastomosis.

Key words: Tubal sterilisation – Refertilisation – Differentiation of tubal epithelium – Ciliated cells – Secretory cells.

Zusammenfassung. Wegen des steigenden Refertilisierungswunsches von Frauen nach voraufgegangener Tubensterilisation sollte tierexperimentell ein Sterilisationsmodell entwickelt werden, bei dem die gesetzten Tubenschäden möglichst gering und die Voraussetzungen für eine Anastomosierung optimal sind.

Dazu wurde in einer ersten Untersuchung bei 30 Kaninchentuben der Tubenisthmus ligiert. Das ligierte Segment wie auch die übrigen Tubenseg-

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mente wurden nach 1 Woche, nach 4 und nach 12 Wochen licht-, raster- und transmissionselektronenmikroskopisch untersucht.

Die wesentlichen morphologischen Befunde bestanden neben einer Einengung der Tubenlichtung in einer Abflachung und in einem Differenzierungsverlust des Schleimhautepithels. Ferner bestand eine leichte Vernarbung der Tubenwand und des subserösen Gewebes im Ligaturbereich. Weiterreichende Schäden wurden nicht gefunden.

Nach Resektion des veränderten Tubensegmentes und nachfolgender Anastomosierung dürften die Voraussetzungen für eine Refertilisierung günstig sein.

Introduction

The increasing number of female patients who desire the re-establishment of fertility indicates the need for a reversible sterilisation method. From our knowledge of the physiology of the oviduct and from clinical experience the rate of success after reconstruction procedures seems to depend on the type and localisation of the tubal damage induced by sterilisation. The present study examines, with the aid of animal experiments, which type of tubal occlusion might be surgically reversible.

The normal structure of the oviduct has been documented by several light and electron microscopic investigations (Nilsson, 1958; Björkman and Fredricsson, 1962; Lehto, 1963; Hashimoto et al., 1962, 1964; Stegner, 1962; Hafez and Blandau, 1969; Kury and Rev-Kury, 1969; Woodruff and Pauerstein, 1969; Odor, 1974; Ferenczy et al., 1974; Critoph et al., 1977; Patek, 1978). Like the endometrium the tubal epithelium undergoes hormonally induced cyclical changes which include microstructural alterations of the cellular organelles (Greenwald, 1958; Nilsson and Rutberg, 1960; Fredricsson and Björkman, 1962; Schultka and Scharf, 1963; Clyman, 1966; Patek et al., 1972; Kugler et al., 1977; Seki et al., 1978). For these reasons these investigations were planned with preoperative hormonal treatment. Some morphological data have been published on changes after tubal ligatures. Marquez-Monter (1972) observed circumscribed serosal fibrosis, atrophy of the muscle layer and cribriform or slitlike constrictions of the isthmic lumina following application of tantal clips to human oviducts. Arnold et al. (1976) reported comparable findings after clipping the ampullary segment but found an almost unaltered mucosa. Diedrich et al. (1977) described inflammation, a hydrosalpinx, and a "reduced" epithelium following applications of plastic clips to rabbit oviducts. Further information concerning morphology after mechanical occlusion of the tube is needed. It is established that ligature of the isthmus offers the advantage of not beeing followed by a hydrosalpinx, in contrast to ampullary occlusion. In addition, the isthmus is especially suitable for a microsurgical anastomosis. Rabbits were chosen for the experiments since their oviducts are comparable in histological structure with the human tube and basic information about the rabbit oviduct is available. Our investigation studied the alterations of tubal mucosa and other tubal wall structures at the ligated isthmus and in the other segments of the

oviduct. Comparative light microscopic, transmission electron and scanning electron microscopic examinations were performed.

Specimens and Investigation Methods

17 fertile female bastard chinchilla rabbits with an age of about 3 months and an average weight of 2,500 g were available for investigation. A flank incision was performed after a short anaesthesia with a mixture of Vetalar and the sedative Rompun. Following careful preparation of the tubal isthmus on both sides an atraumatic purse-string suture was done with a 5XO-silk-thread, under slight traction. Postoperatively the rabbits received a subcutaneous injection of 1 ml chloramphenicol daily for three days.

In four rabbits the oviducts en bloc, or the ligated segment alone were removed one week after the ligature. In five animals the excision was performed after four weeks and in six after twelve weeks. Two animals with sham operations served as controls. The rabbits were given a subcutaneous injection of 2.500 I.U. HCG 48 h before the removal of the oviducts or the ligated tubal segments.

For perfusion fixation the abdominal aorta was cannulated above the renal arteries, washed with isotonic NaCl-solution for two minutes and then perfused with 2% buffered glutaraldehyde, maintaining a pressure of 90–120 mm Hg. Later preparation of the excised tube or tubal segment was done in a bath of glutaraldehyde. The following segments of the oviducts were examined: Fimbriae, ampullae, ampullary-isthmic junction (AIJ) and the ligated isthmic part. Each segment measured about 3–5 mm. Control segments were postfixed in 10% neutral formalin; paraffin sections were stained with haematoxylin-eosin, iron-haematoxylin-picrofuchsin according to van Gieson, combined with resorcine, and the Alcian-PAS-reaction. The other specimens were postfixed in 1% buffered osmium tetroxide and embedded in Araldite. Semithin sections of the Araldite blocs were stained with methylene blue. Up to 50 sections were cut from the area around the ligature and up to 10 sections were examined from the other tubal segments. For scanning electron microscopy the excised segments were spread, dehydrated and dried according to the critical point procedure. Following vaporising with gold these specimens were examined with the aid of a JSM-U3-SEM (JEOL). For transmission electron microscopy Araldite thin sections were treated with uranyl acetate and lead citrate; examination was done in the Siemens Elmiskop 101.

Light Microscopic Findings

Eight out of 30 ligated oviducts did not show a definite luminal stenosis. Correspondingly, the findings described below were either not at all evident or were limited to discrete inflammatory alterations of the outer tubal wall. This "negative" result was seen once in the first group (1 week following ligature), twice in the second group (4 weeks following ligature) and five times in the third group (12 weeks following ligature). These 8 tubes will not be further considered in the presentation.

At one week following surgery the ligated segment shows haemorrhage around the ligature in both the outer tubal layers and in the mucosa. Superficial to the mucosal bleeding partial desquamation of the epithelium is observed. The intact epithelium starts to flatten and to loose its cellular polarity; anisometric, partially vesicular nuclei with prominent nucleoli are seen in comparison with the normal histology (Figs. 1, 2). Singular mitoses occur (Fig. 3a, b, c) and several epithelial cells are polynuclear. The cilia often appear shortened and are reduced in number. Small intraepithelial cysts are surrounded by cilia. Peg cells – characterized by dark cytoplasm – are seen next to epithelial cells

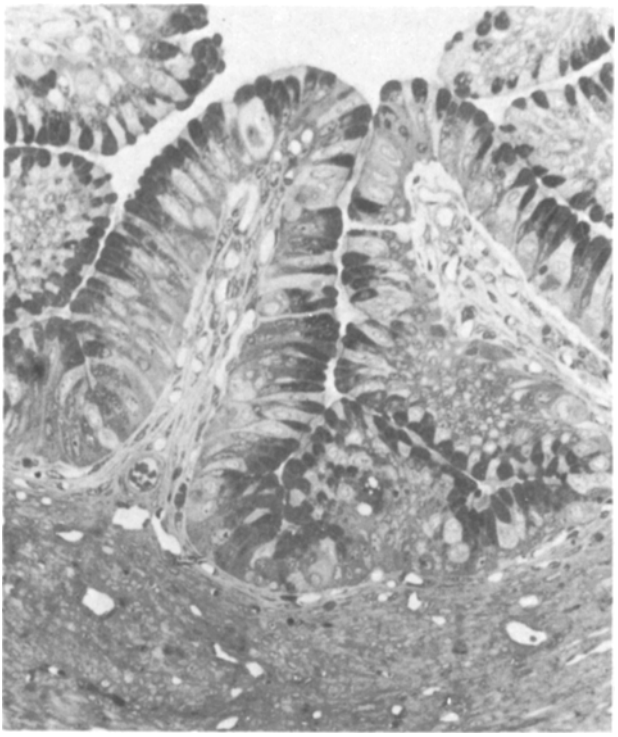

Fig. 1. Normal tubal mucosa in the isthmic segment with closely packed secretory cells. Semithin section, methylene blue, 190 ×

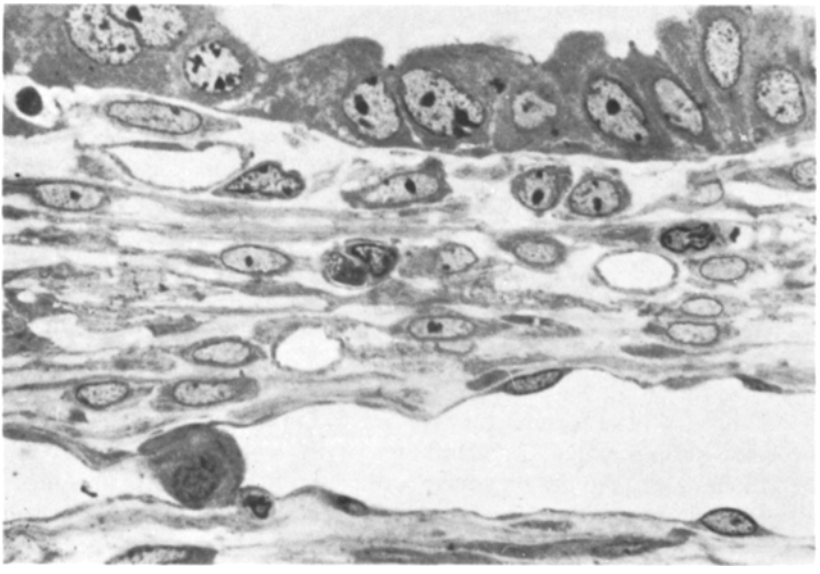

Fig. 2. Flattened epithelium with anisometric large, pale nuclei with large nucleoli. Oedema of the submucosal layers of the tubal wall. One week following ligature of the tubal isthmus. Semithin section, methylene blue, 1,000 ×

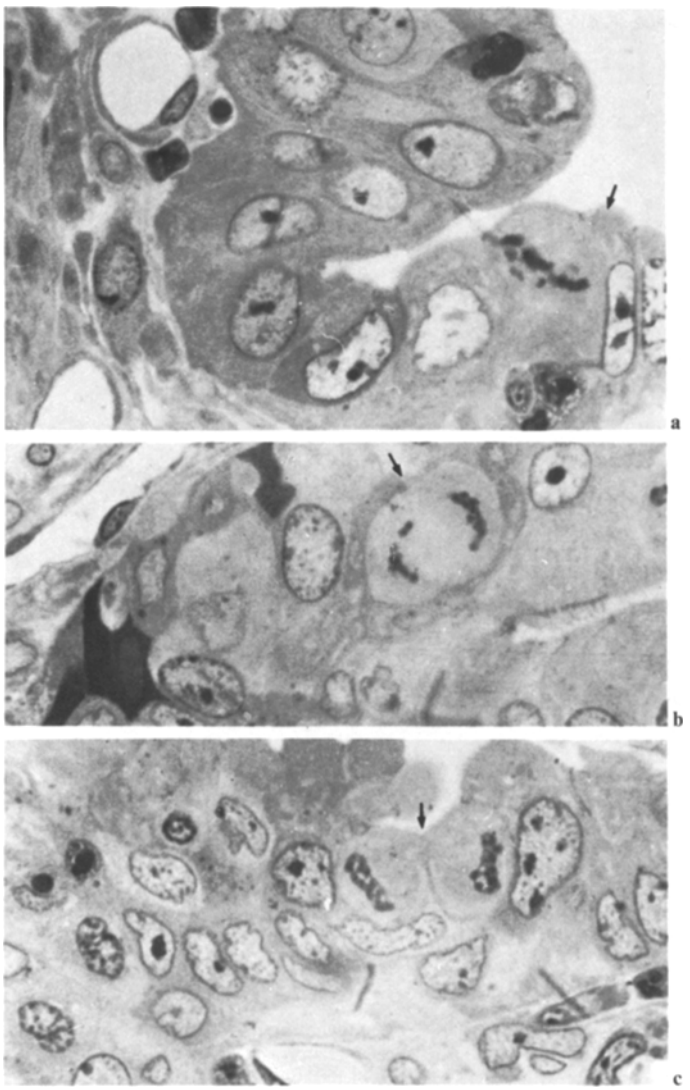

Fig. 3a–c. Mitotic figures within epithelial cells, one week following ligature of the tubal isthmus. Semithin section, methylene blue, 1,000 ×

with some secretory granules (Fig. 4a). The intact epithelium is penetrated by singular granulocytes and the oviduct muscle layers show a spotty oedema. Some muscle cells contain nuclei which appear to be dying. Lymphocytes and granulocytes can be seen between smooth muscle cells, and here and there the outer tubal layers are replaced by inflammatory granulation tissue with granulocytes, lymphocytes and histiocytes or foam cell granulomata. At the AIJ one centimeter distant from the ligature, discrete haemorrhages and loss of the cellular polarity may appear within the epithelium. Some of the epithelial

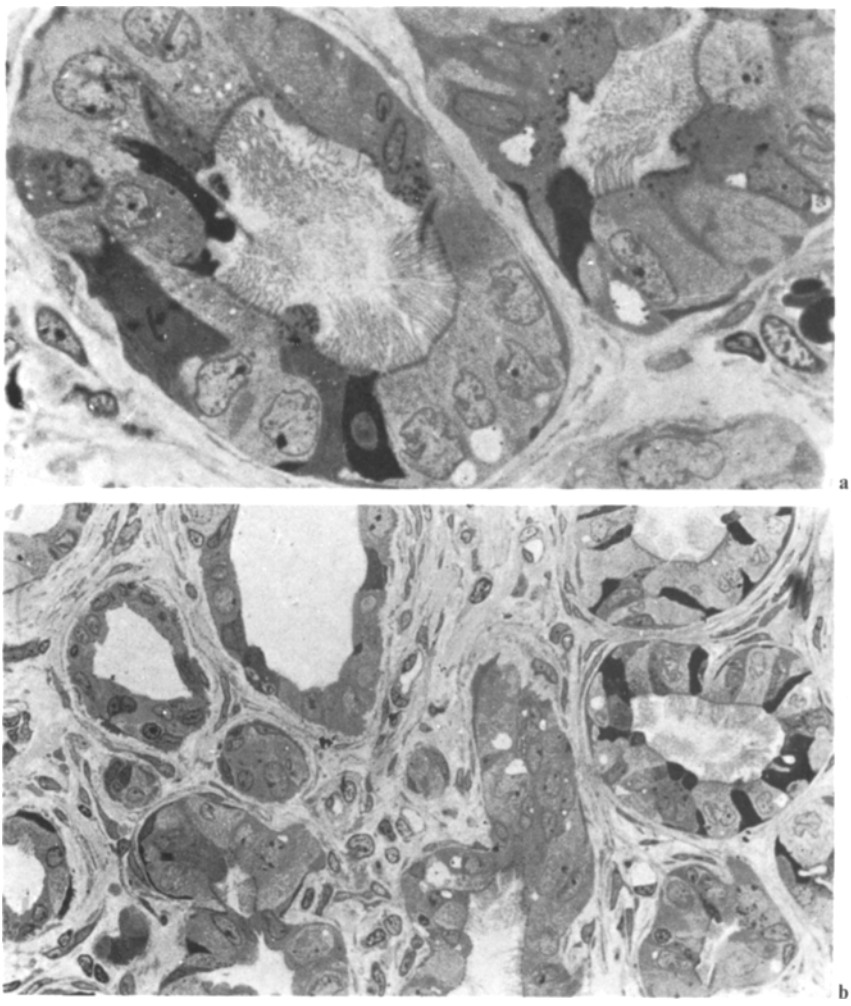

Fig. 4. a Peg cells in the isthmic mucosa, one week following ligature of the isthmic segment. Semithin section, methylene blue, 1,000×. **b** Cribriform transformation of the tubal lumen; 4 weeks following ligature of the isthmic segment. Semithin section, methylene blue, 750×

cells contain swollen nuclei and the subepithelial stroma includes some lymphocytes next to dilated capillaries. Occasionally comparable changes occur in the ampullary segment. The other tubal wall layers of the AIJ and the ampulla are not altered, apart from focal bleeding in the subserosal tissue. Morphological alterations of the fimbriae were not recognized during light microscopical examination, although some dilated lymphatics are found in the fimbrial stroma.

Four weeks following tubal ligature the tubal lumen is often transformed into a network so that some small luminal spaces, lined by tubal epithelium, develop in the region of the ligature (Fig. 4b). In other cross sections the lumina contain epithelial debris. Mitoses are rare. An increased number of peg cells

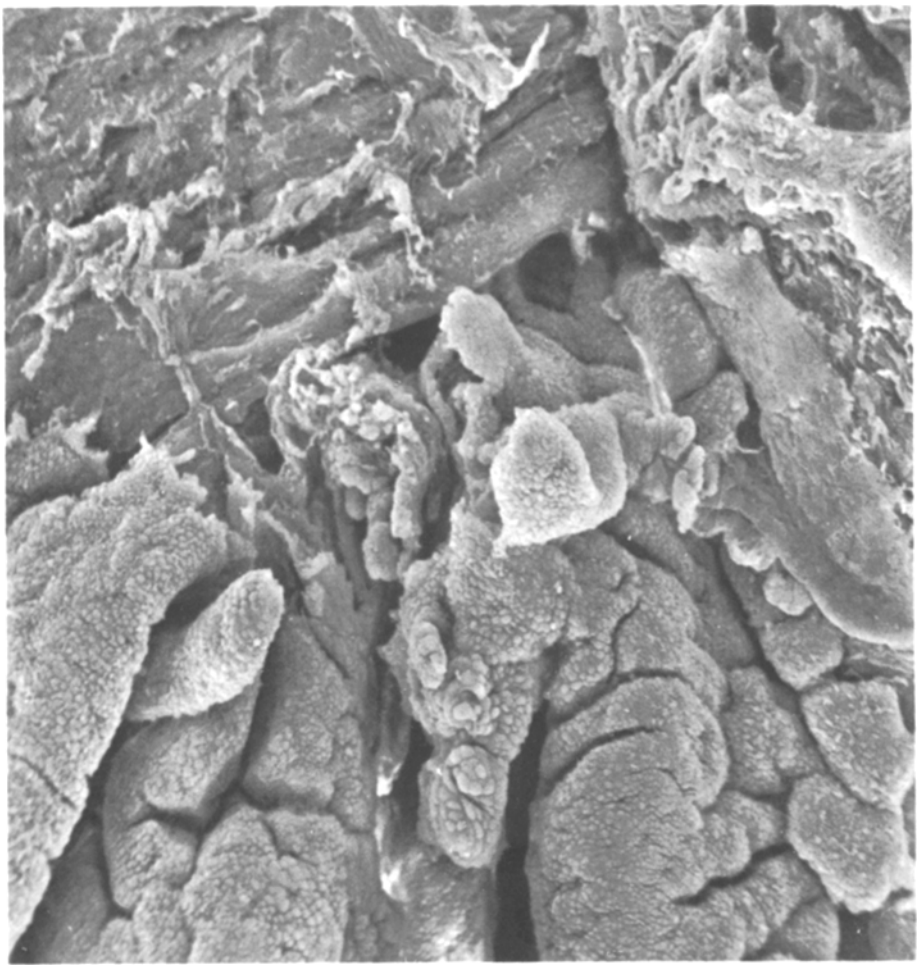

Fig. 5. Scanning electron microscopic surface view of the constricted mucosa with an adjacent mucosal defect (*top*) at the isthmic segment; four weeks following ligature of the isthmic portion. 180×

are constantly seen. Although a partial or total loss of cilia can be seen at the surface, ciliated cysts appear in increasing numbers. Different stages of necroses are observed within the muscle layer. Single granulocytes, lymphocytes and macrophages penetrate all of the tubal wall layers. Next to the ligatures thread granulomata are developed, sometimes surrounded by calcified material and small mesothelial spaces. Here and there dilated lymphatics are evident within the outer layers. At the AIJ and the ampullary segment the epithelium is intact and the other tubal layers are free of necrotic lesions. Single granulocytes and lymphocytes are seen in the subepithelial stroma and in the outer layers of these tubal segments. The fimbriae are not altered.

Twelve weeks after the ligature mucosal folds no longer exist in the area of the ligature. The tubal lumen is constricted, measuring about 100 μ in smallest

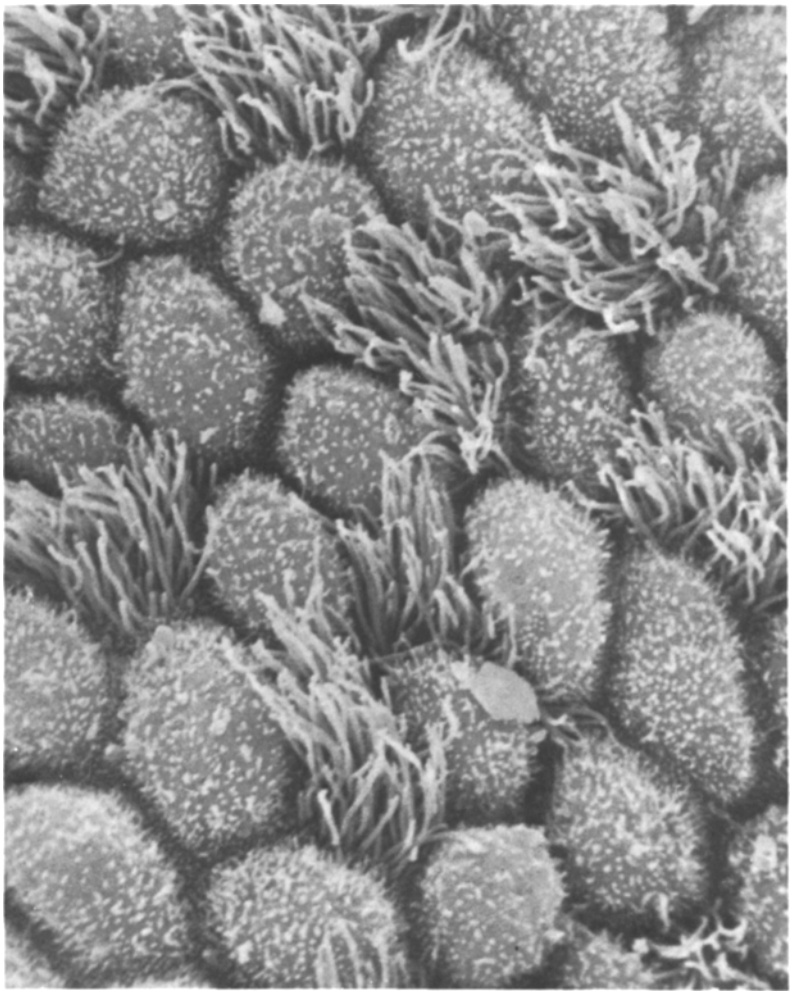

Fig. 6. Scanning electron microscopic survey of the normal isthmic mucosa with numerous cilia between secretory cells. 3,600 ×

diameter. The epithelium is often flattened with loss of cilia and secretory granula. Epithelial debris may be found within the lumen. Peg cells are multiplied, and ciliated intraepithelial cysts are increased. Granulocytes and lymphocytes are seen in the mucosal stroma. Single macrophages within the epithelium are laden with cellular debris. Fibrosis is seen in the muscle layer and in some parts of the mucosal stroma. Some blood vessels in the subserosa are constricted by fibrosis. Complete obliteration of the tubal lumen had not developed by this time. There were no alterations of the wall layers at the AIJ, the ampulla and the fimbriae.

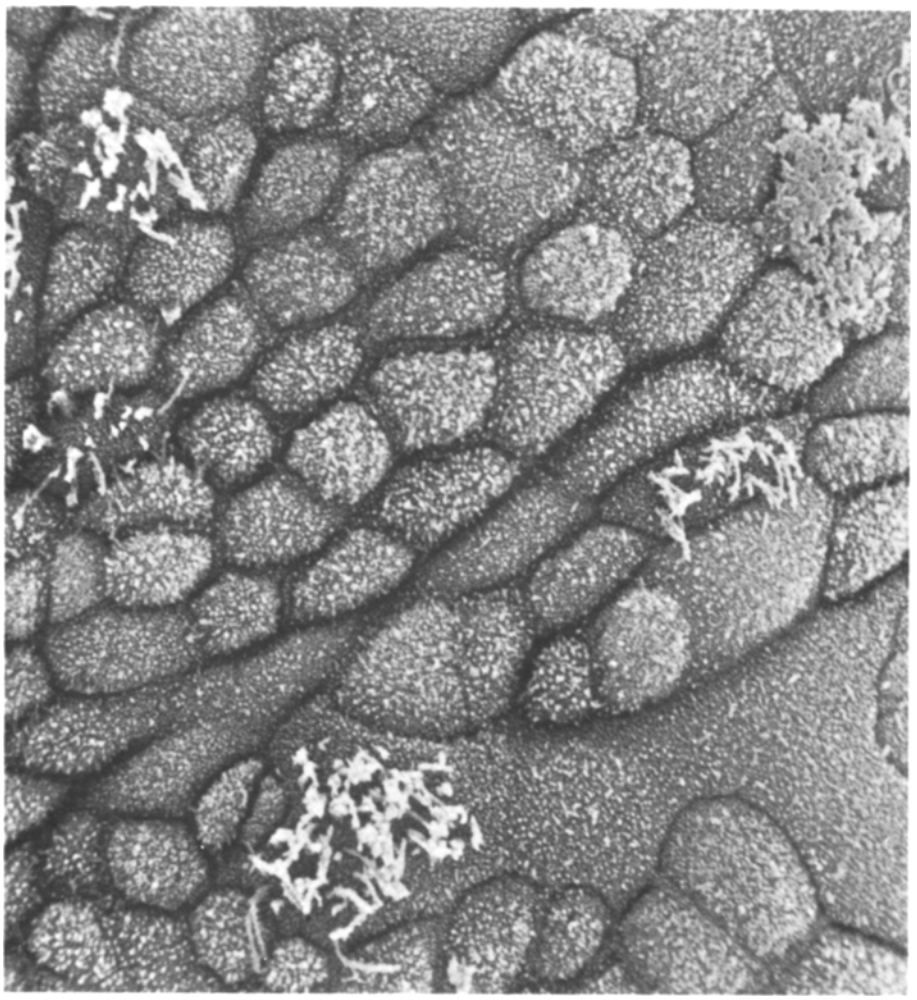

Fig. 7. Flattened isthmic epithelium with reduction of cilia; 12 weeks following ligature of the isthmus. 3,600 ×

Scanning Electron Microscopic Findings

There are significant alterations at the area of the ligature only. In this region compressed mucosal folds with epithelial damage and desquamation of epithelial cells appear, as described under light microscopy (Fig. 5). In comparison with the normal mucosal surface (Fig. 6) the epithelium of the constricted part is flattened (Fig. 7). The cellular surfaces vary in size, are rounded or even polygonal. Cilia are almost completely lacking on some folds. Here and there solitary ciliated cells with some shortened processes persist. The adjacent mucosal seg-

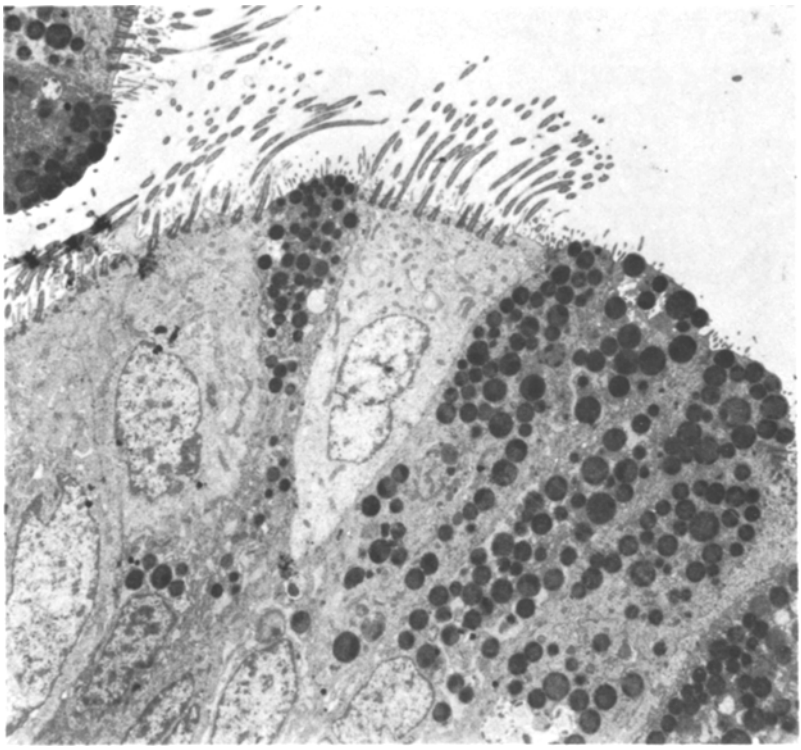

Fig. 8. Transmission electron microscopic aspect of the normal isthmic epithelium with ciliated cells and densely granulated secretory cells. 3,600 ×

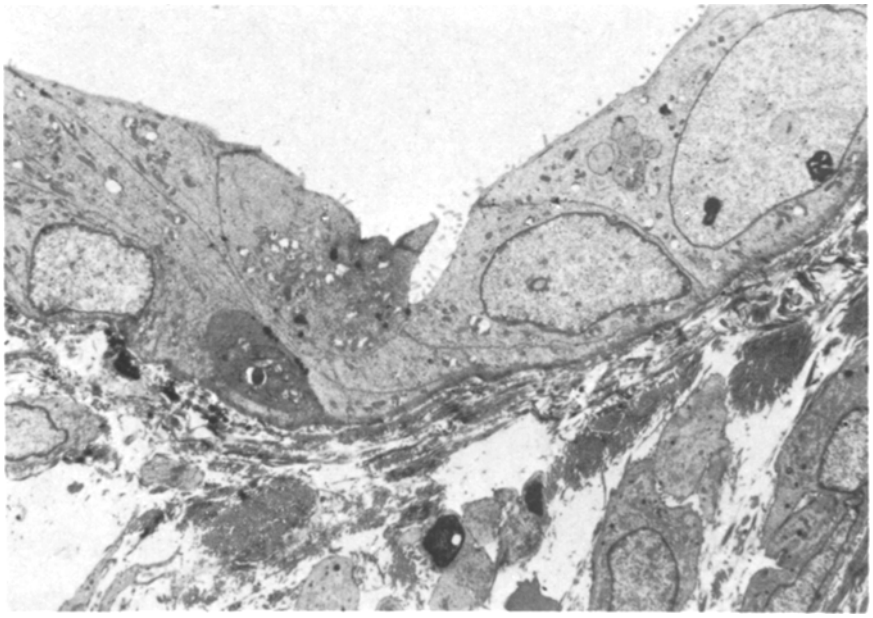

Fig. 9. Flattened isthmic epithelium with loss of cilia; one week following ligature of the isthmic segment. 4,500 ×

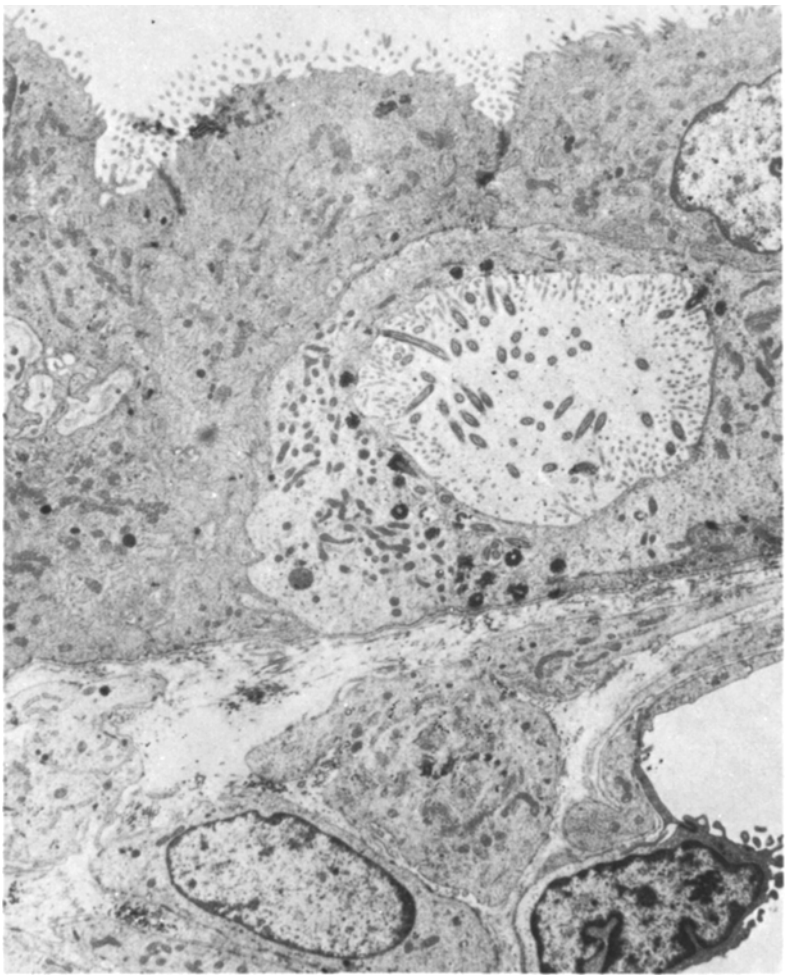

Fig. 10. Small intraepithelial ciliated cyst and loss of cilia at the epithelial surface. 2,500 ×

ments, 1 or 2 mm distant from the ligated part, are not pathologically altered except for moderate reduction of the number of ciliated cells.

Transmission Electron Microscopic Findings

1 week following the ligature of the isthmus the epithelium of this area is considerably flattened (Fig. 8, 9). There are no cilia on the mucosal surface but there appears to be an increased development of intracytoplasmic cilia, forming small ciliated cysts (Fig. 10). The epithelial microvilli are found at variable density, and the mitochondria are often swollen. Desquamation figures of degenerating epithelial cells are noticed. Close to the ligature almost no secretory cells are found. The peg cells are characterized by dark cytoplasm

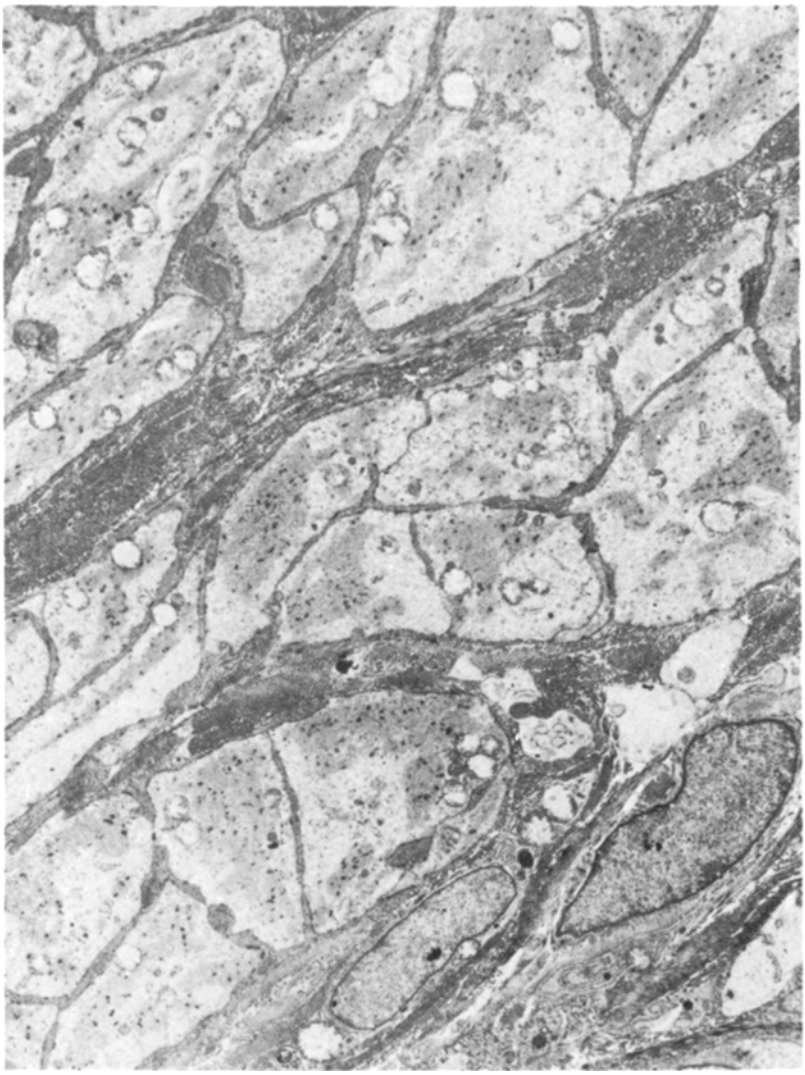

Fig. 11. Acute necroses of tubal muscle cells; four weeks following isthmic ligature. 4,500 ×

with condensation of the cytoplasmic ground substance, they contain just a few cellular organelles and their nuclei show a dense chromatin and different stages of pyknosis. The mucosal stoma is loosened by oedema. The other alterations correspond to those found by light microscopy.

The findings described above are partly developed at the AIJ as well. In this segment epithelial necroses and cellular desquamation may also be observed during the early phase. Ciliated cells with reduced numbers of cilia can also be seen without significant flattening of the epithelium. Here and there the intercellular spaces are markedly dilated. The mucosa of the ampullary region and the fimbriae shows normal cytological structure.

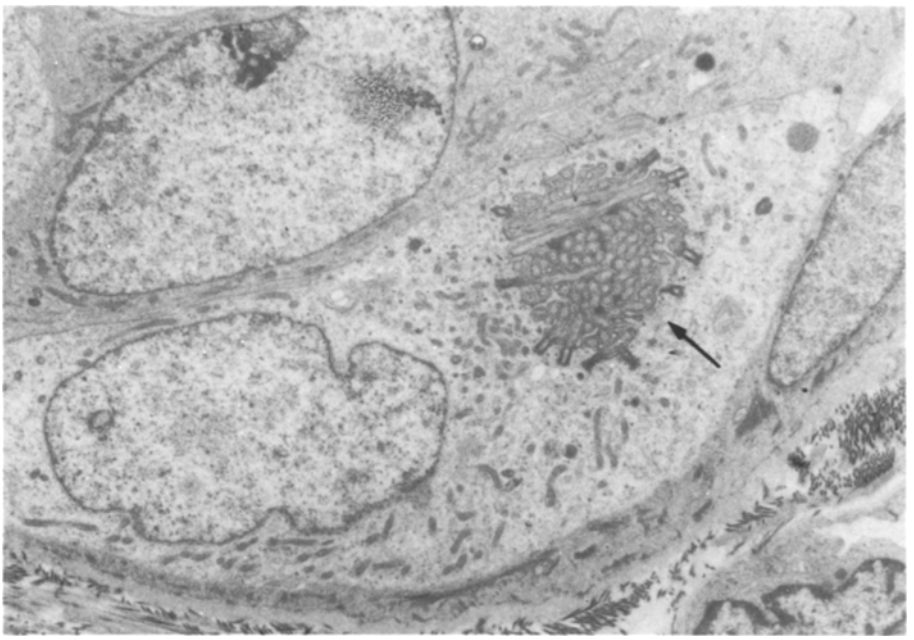

Fig. 12. Tubal epithelial cell with an intensive intracytoplasmic ciliogenesis (*arrow*). 6,000 ×

Four weeks after ligature most of the epithelial cells are flattened in the vicinity of the ligated part. Cilia and secretory granula are almost absent. Some cells contain two nuclei. Within the other wall layers, degenerating smooth muscle cells are obvious (Fig. 11) showing dying nuclei, disrupted cellular membranes and loosening of the cytoplasmic ground substance. Because of the almost normal findings 1 week after ligature and the minor alterations found in the light and the scanning electron microscopes, transmission electron microscopy of the other tubal segments was not performed.

Twelve weeks after the ligature there was no essential change in the cytological appearance at the area of the ligature. Occasionally an increased formation of intracytoplasmic cilia could be demonstrated preceding the development of ciliated cysts (Fig. 12). The epithelial cytoplasm appeared lightened or poor in organelles. In one case a thickening of the garland-like convoluted basal membrane was observed (Fig. 13). The other findings correspond to those described for light and scanning electron microscopy.

Discussion

Comparative light microscopic, scanning and transmission electron microscopic investigations of the rabbit oviduct following ligature of the isthmic part show that the tubal wall alterations are mostly limited to the area around the ligature. Gross alterations such as the development of hydrosalpinx, degeneration of ampullary or fimbrial epithelium or obliteration of other segments did not

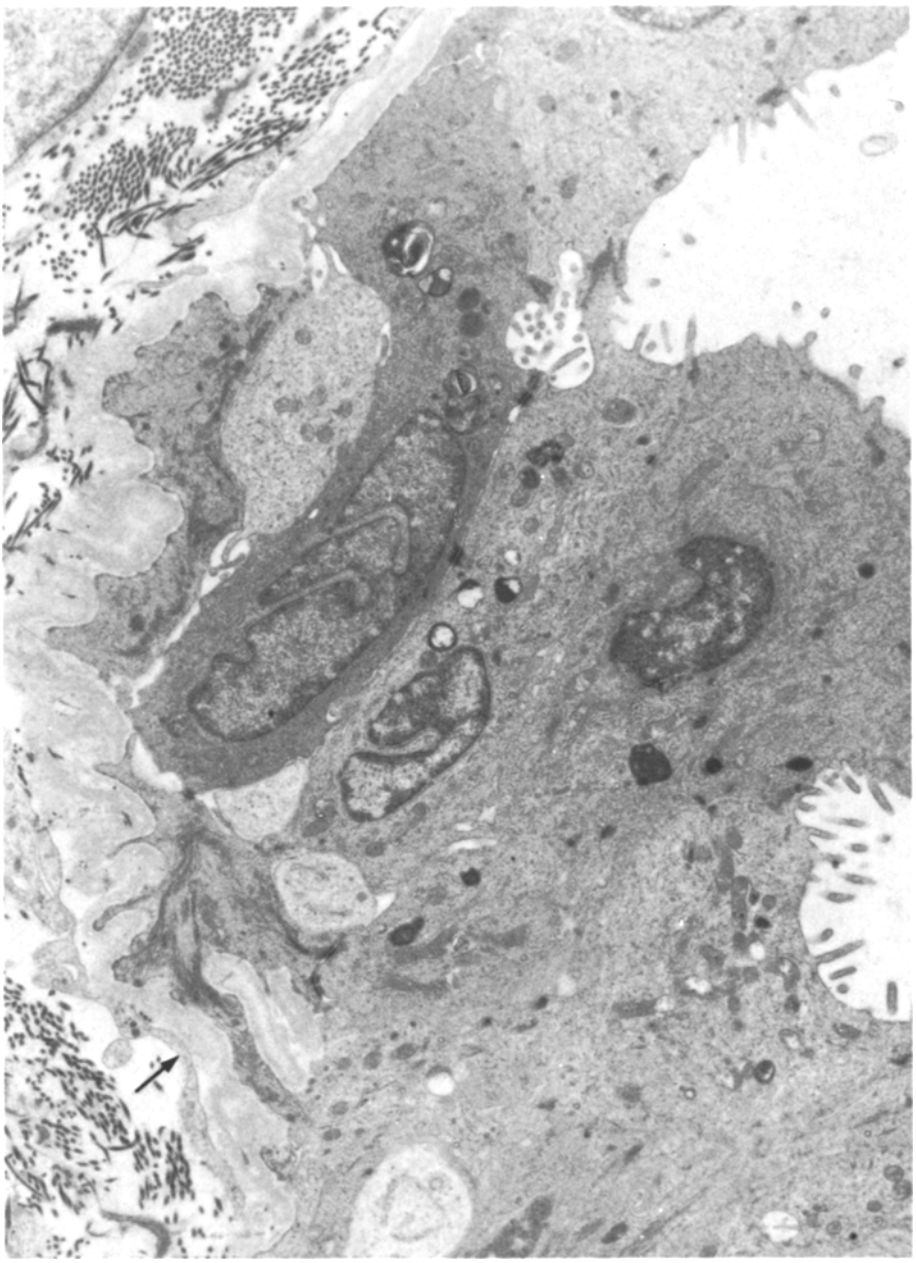

Fig. 13. Tubal epithelium with partial loss of differentiation and formation of myelin figures above a thickened basal membrane (*arrow*). 9,000 ×

occur. The findings demonstrated may be caused in part by local disturbance of the blood supply in the ligated area, suggested by mitochondrial swelling in intact cells of the tubal wall. In addition to the hypoxic lesions due to the compression of the local blood vessels direct injury to the cells may occur. The development of granulation tissue with foam cells in the surroundings of the threads indicates the possibility of a direct mechanical lesion, at least for the outer tubal wall layers. The findings in the adjacent segments mainly represent bland tissue reactions induced by operative trauma, they have vanished by 4 weeks later. The degree of the pathological alterations seen seems to depend on the severity of tissue compression and subsequent, relatively discrete, granulomatous inflammatory reaction. In 8 oviducts the ligatures were not tied properly; thus the important compression effect was missing. However, it seems as if this fault cannot always be prevented and it is not possible to calibrate the tension used in tying the knot. Ligatures drawn too tight led to cutting of the full thickness of the tubal wall during initial experiments.

Constrictions of the lumen and cribriform transformations of the cross sectional picture, with formation of spaces lined by normal or flattened epithelium, are limited to the ligated segment. The cribriform transformation may be explained by adhesions and subsequent fibrous fusion of stromal septa after desquamation of the covering epithelium within the constricted area. Fibrosis of this tubal segment corresponds to the replacement of necrotic tubal muscle cells by connective tissue. Similar changes were described by Marquez-Monter (1972), who examined the reaction of 47 human oviducts following application of tantal clips to the isthmic segment. Apart from cribriform or slitlike constrictions of the lumina Marquez-Monter found complete obliteration in 8 cases. During follow up of 3 months there was no instance of complete obliteration of the tubal lumen in the present experiments. In all cases a small residual lumen persisted, measuring about 100 μ in smallest diameter. For comparison, the diameter of a mature ovum of a rabbit measures 180 μ.

The essential morphological alterations, including constrictions of the lumina, flattening of the epithelium to a varying degree and occasional cribriform transformation of the lumen, were observed at the ligated isthmus. Cytologically the alterations included a loss of epithelial cellular differentiation which resulted in a decrease of cilia and a reduction in the number of secretory granules. At the ligated segment flat pale cells, poor in organelles, persisted. Synchronously with the reduction of cilia on the mucosal surface an increase of intracytoplasmic ciliated cysts was observed. These cysts are regarded as the morphological marker of ciliogenesis by the most investigators (Mihalik, 1934; Hamperl, 1950; Overbeck, 1969). Hamperl emphasised that cilia are probably formed by this way in all comparable mucosal membranes. In contrast to this opinion Jirsova et al. (1977) interpreted the cysts as regressive alterations. We do not want to discuss the general meaning of ciliated cysts in epithelial cells, however, the increased development of ciliated cysts within the altered epithelium and in those cells with loss of cellular differentiation, suggests that the cysts should be regarded as an expression of disturbed or unphysiological ciliogenesis.

The alterations of the tubal mucosa described above emphasise the fact that complete obstruction or severe constriction of the tubal lumen is not the

only reason for blockade in the migration of the oocyte. Loss of cilia and secretory function of the tubal mucosa along a distance of 3–5 mm may alone have a blocking effect. This is supported by the animal experimental findings of Diedrich et al. (1977). 2–12 weeks after application they removed plastic clips from the oviducts of 12 rabbits and although these animals still had patent oviducts fertility could not be restored. This result seems to demonstrate that tubal function must have been impaired by the effect of the applied tubal clamp.

The importance of the cilia for the migration of the ovum is underlined by numerous reports. Odor (1973) observed an interruption of oocyte migration in rabbits if the number of ciliated cells dropped below 44%. The same result was published by Brosens (1975) who counted the ciliation index of human fimbriae. Critoph (1977) observed a rise in the beating rate of cilia during the postovulatory phase of the cycle. In the ampulla there was a rise of 16%, in the isthmus of 18%. Critoph believes that his findings demonstrate the importance of the cilial function for even isthmic ovum transport. Koester (1969), who performed investigations on the hormonal regulation of tubal secretion and its influence on the migrating oocyte, underlined the importance of the secretory cells in the isthmic part of the oviduct.

The findings presented show that after ligature of the tubal isthmus alterations of the ciliated and secretory cells are limited to the area close to the ligature. Resection of the altered isthmic tubal segment and subsequent anastomosis promises good conditions for restoration of fertility. The aim of further investigations is to find out the chances of reversal and to compare these findings with those following ligature and anastomosis in the ampullary segment.

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