Neovaginal mucosa after Vecchietti’s laparoscopic operation for Rokitansky syndrome: Structural and ultrastructural study

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Objective: This study was undertaken to evaluate structural and ultrastructural characteristics of the mucosa of neovaginae created by Vecchietti’s laparoscopic operation for Rokitansky syndrome.

Study design: Vaginoscopy and Schiller test were performed 3, 6, and 12 months after the operation in 106 patients. A biopsy specimen of the neovagina obtained 12 to 18 months after surgery in 19 patients was examined by light, scanning electron, and transmission electron microscopy.

Results: At vaginoscopy, the neovaginal mucosa appeared smooth, lacking the folds that characterize the normal vagina; 12 months after the operation, an iodine-positive epithelium was present in all neovaginae. Mild ultrastructural modifications, as compared with normal vaginal mucosa, were reduced maturation, inflammatory infiltration, and tendency to superficial desquamation.

Conclusion: At a 12-month follow-up, the mucosa of neovaginae created by the Vecchietti technique is comparable to the normal vaginal mucosa, with mild structural and ultrastructural modifications that we believe might be due to reduced vascularization.

Mayer-Rokitansky-Kuster-Hauser syndrome is a rare congenital anomaly of the female genital tract characterized by normal external genitalia, absence of the vagina and uterus, and normal development and function of the ovaries. The treatment of this condition consists in the creation of a neovagina, to allow sexual intercourse. Among the numerous techniques for creating a neovagina that have been proposed, Vecchietti’s operation and its laparoscopic modification have been proved safe and effective. The Vecchietti’s technique consists of placing 2 threads that course subperitoneally, cross the vesico rectal space, and connect a traction device placed suprapubically with an acrylic olive placed in the vaginal dimple. The constant traction exerted on the olive by the traction device creates a deep...
invagination of the vesicorectal space in 7 to 8 days. The daily application of apposite dilators for the following weeks allows the creation of a neovagina comparable in size to a normal vagina.

A possible advantage of Vecchietti’s approach, in comparison with other techniques for the creation of a neovagina that use heterotopic organs such as myocutaneous flaps or bowel segments, is that the neovagina is coated by a smooth, nonsecreting epithelium similar to the normal vaginal epithelium. In the current study, we sought to evaluate vaginoscopic, structural, and ultrastructural characteristics of the mucosa coating the neovagina obtained with the Vecchietti’s laparoscopic operation.

Material and methods

From 1993 to 2004 we have performed the laparoscopic creation of neovagina with Vecchietti’s technique in 106 patients with Rokitansky syndrome. Mean age was 16.9 years (range 15-34). All patients used vaginal dilators in the 4 to 6 weeks after the operation and all had sexual intercourse within 1 year from the creation of the neovagina. The patients were followed up for a period varying from 1 to 9 years. The study was approved by the Institutional Review Board.

A vaginoscopic evaluation was performed 3, 6, and 12 months after the operation. Vaginoscopy was performed with a laparoscopic 0-degree optic, with an assistant retracting the neovagina using 2 small lateral retractors. During the procedure, the neovagina was accurately cleaned with saline solution and a Schiller test, ie, the application of Lugol’s iodine, was performed.

A biopsy specimen of the neovagina was obtained 12 to 18 months after the operation in the 19 patients who gave their consent to the procedure. Serum estradiol concentrations were normal in all patients either before the operation or at the time of the biopsy. Biopsy specimens were obtained from the midthird of the lateral wall of the neovagina. Each specimen was subdivided into fragments and then prepared for examination by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Tissue fragments for LM were fixed in 10% buffer formalin and paraffin embedded; serial sections were obtained that were stained with hematoxylin and eosin. The tissue samples for SEM, after abundant washing in saline solution, were fixed for 12 hours in 5% glutaraldehyde and cacodylate buffer, pH 7.4; the preparations were then dehydrated with increasing concentrations of alcohol (30% to absolute) and acetone, subjected to critical point drying and gold sputter coating, and observed with a SEM Cambridge stereoscan 150 (Altran Corporation, Boston, MA). The tissue samples for TEM were fixed in 2.5% glutaraldehyde in phosphate buffer for 2 hours, then washed 3 times in the same buffer, postfixed for 1 hour in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol (25% to absolute), and embedded in epoxy resin. The ultrathin sections were stained with uranyl acetate and lead citrate. The preparations were observed with a TEM Jeol JEM 1010 (Jeol Ltd, Tokyo, Japan).

Results

Three months after surgery, all the neovaginae were at least 6 cm in depth with a transverse diameter, upon
distension with 2 fingers, of at least 4 cm. At visual inspection by vaginoscopy, the mucosa was pink and smooth, lacking the folds that characterize the normal vagina. At the Schiller test, the epithelium proved to be iodium-positive with limited iodium-negative areas that progressively reduced in size in the course of the follow-up, demonstrating the maturation of the glycogenic activity of the epithelium of the neovagina. In particular, the epithelium demonstrated a capacity to colonize the neovagina from the introitus to the apex, coating 80% of the neovaginal surface at a 3-month follow-up and 90% at a 6-month follow-up; the neovagina was fully coated in all women 12 months after the operation (Figure 1).

At LM evaluation, the vaginal mucosa appeared normal in thickness and with aspects of glycogen accumulation. The epithelium showed a lower degree of maturation and increased desquamation of superficial cells compared with normal vagina. Areas of parakeratosic hyperkeratosis were also present. A mild infiltration of inflammatory cells both acute and chronic was always observed, in some cases reaching the epithelial layer (Figure 2).

At SEM evaluation (Figure 3), the neovaginal mucosa was flat. The transverse vaginal folds or “rugae,” determined by the presence of longitudinal smooth muscle cells and venous plexous under the mucosa of the normal vagina, were poorly represented. The surface epithelium consisted of flattened polygonal cells with thin indented margins. The typical desquamating appearance of normal vagina, determined by the frequent lack of connections between cells’ margins, was less evident in

**Figure 2**  Histologic characteristics of a A, neovagina and B, a normal vagina (hematoxylin-eosin stain, original magnification ×100). See text for details. In the circles: inflammatory cells infiltrating the epithelium of the neovagina.

**Figure 3**  Ultrastructural characteristics (SEM) of A, a neovagina (scale bar = 20 μm) and B, a normal vagina (scale bar = 10 μm). The surface epithelium of the neovagina consists of flattened polygonal cells with indented margins. The absence of folds or “rugae” gives to the neovaginal epithelium a flat appearance. The intercellular margins of the neovaginal epithelial cells are thinner and flatter.
the neovaginal mucosa caused by the presence of thin and flat intercellular margins. However, where the lack of connections between cells of neovaginal mucosa was observed, zipper-like connections were well represented. Longitudinal and concentric microridges were less homogeneously distributed, more flat and sometimes absent in the neovaginal epithelium, compared with the epithelium of the normal vagina. Some areas were covered by fibrillar material encompassing erythrocytes, attesting bacterial cytolysis; this is a typical characteristic of glycogen-containing epithelium, which is in fact well represented in the neovaginal epithelium, both in a diffuse form and in cytoplasmic “lakes.” In some neovaginae we observed areas of disepithelization with inflammatory infiltration of the submucous layer; in these areas, the submucous connective tissue with mildly protruding longitudinal architecture reached the surface.

TEM demonstrated morphologic characteristics ranging from normality to mild modifications. In most samples we found well-preserved epithelium showing normal basal layer with abundant hemidesmosomes toward continuous basement membrane with normally distributed anchoring fibrils. Only few samples showed focal ultrastructural modifications in the basal layer of the mucosa: some cells showed cytoplasmic vacuoles, loss of mitochondrial cristae, and basement membrane abnormalities such as thinning, focal interruption, and irregularly distributed anchoring fibrils (Figure 4). Rare basal cells presented nuclear chromatin condensation.

Figure 4  Ultrastructural modifications of the basal layer and nearby extracellular matrix (ECM) shown by TEM in neovaginal mucosa. 

- **a, A.** Normal basal layer showing abundant hemidesmosomes (white arrow) toward continuous basement membrane (BM) with normally distributed anchoring fibrils (arrows).
- **b, B.** Cytoplasmic vacuolization (arrows).
- **B.** Loss of mitochondrial cristae (white arrow), BM thinning (arrowhead) and irregularly distributed anchoring fibrils (arrows).

**a, b,** Scale bar = 2 μm. **A, B,** Scale bar = 1 μm.
Comment

The current study demonstrates that at a 12-month follow-up the mucosa of the neovagina created by the Vecchietti technique is very similar to the normal vaginal mucosa.

This study was performed on a limited number of patients, despite the high number of operations performed, because most of the women refused to undergo a biopsy for the sole purpose of scientific research. For the same reason we could not obtain serial biopsy specimens from the same patient, thus limiting information on the maturation of the epithelium of the neovagina. Despite such limitations, we believe that the strength of our study consists in the comprehensive evaluation of the neovaginal mucosa, including structural and ultrastructural characteristics. Serial vaginoscopic evaluations demonstrate that the epithelium of the vaginal introitus colonize the whole cavity of the neovagina in a 6- to 12-month period. The mucosa of the neovagina has a glycogenic capacity that progressively extends from the introitus to the vaginal apex, as demonstrated by the Schiller test. The main difference of a neovagina compared with a normal vagina is the absence of folds, as the mucosa of the neovagina is flat and smooth, whereas the normal vagina in young women is rough and folded. At light microscopic evaluation, the neovaginal mucosa showed mild modifications compared with normal vagina represented by reduced maturation, inflammatory infiltration, and tendency to superficial desquamation. At EM evaluation, most samples had normal ultrastructural characteristics. Mild differences between the neovaginal mucosa and the mucosa of a normal vagina were more evident at SEM evaluation, showing a flat neovaginal mucosa lacking the transverse folds that characterize normal vagina. This was determined by the paucity of smooth muscle cells and venous plexus under the mucosa of the neovagina. Inflammatory infiltration of the submucosal layers, areas of desepithelization, and protrusion of the submucous connective tissue were also observed. On the other hand, glycogen was well represented in the neovaginal epithelium. TEM evaluation showed, in few samples, focal changes in the basal layer such as cytoplasmic vacuoles, loss of mithocondrial cristae, and basement membrane abnormalities. In our opinion, the mild differences between neovaginal and normal vaginal epithelium observed at LM and EM evaluation might be due to a reduced vascularization and, to a lesser extent, an inflammatory condition of the newly formed vaginal mucosa.

To date, there has been limited consideration of the characteristics of the mucosa coating the neovaginae obtained with the different techniques proposed for the treatment of Rokitansky syndrome. The 2 previous studies describing the epithelium of the neovaginae created with the Vecchietti technique evaluated only the cytologic findings. The first study was performed during the first and second month after surgery and showed the presence of inflammatory and parakeratosis cells. The other study, performed 2 to 12 years after surgery, demonstrated that the mucosa of neovaginae obtained with the Vecchietti technique is completely similar to the mucosa of normal vagina, including the capacity of responding to hormonal stimuli.

Most of the techniques proposed for creating a neovagina consist in the surgical creation of a tunnel between the bladder and the rectum, which is then coated with the transplantation of homologous tissue. Studies evaluating neovaginae created with the McIndoe technique, ie, with dermoeppidermic flaps, have been conducted by using both LM and SEM and demonstrated that homologous human skin transplanted into vaginal areas retains most of its morphologic and histochemical characteristics, namely, keratinized epithelium with sebaceous glands and hairs, even 12 years after the operation. Similarly, neovaginae created with intestinal segments present a mucous-secreting epithelium that maintains its characteristics for numerous years. The epithelium of neovaginae coated with the peritoneum of the pouch of Douglas, according to the technique proposed by Davydov, was studied at LM in 23 patients by means of microbiopsy specimens obtained 70 days after the operation: all specimens showed stratified squamous metaplastic epithelium containing glycogen in the superficial squamous layers. The homotransplantation of amniotic membrane has also been proposed to line the surgically created vesicorectal tunnel: the ultrastructural evaluation with TEM of the neovaginae obtained with this technique showed that the amniotic membrane is able to complete metaplasia into squamous cells, but the mechanisms of this cellular transformation is unknown.

In conclusion, the Vecchietti’s operation allows the creation of a neovagina coated with a mucosa that is almost identical to that of a normal vagina. The presence of a glycogen-secreting epithelium is the biologic basis for the creation of a habitat and a function similar to those of a normal vagina (ie, presence of lactobacilli, production of lactic acid, normal vaginal pH of 4.5 or less). Our study adds another element in favor of the Vecchietti’s operation, that was already known to be a simple, effective, and safe procedure.

References

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