American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 05 PAGES: 63-80
SJIF IMPACT FACTOR (2022: 5. 705) (2023: 6.534) (2024: 7.7)
OCLC – 1121105677
Publisher: Oscar Publishing Services

Journal Website: https://theusajournals.com/index.php/ajbspi

Research Article

REACTIVE CHANGES IN THE CELLS OF THE MUCOUS MEMBRANE OF THE ARTIFICIAL VAGINA IN PATIENTS WITH AGENESIS OF THE GENITAL ORGANS

Submission Date: May 21, 2024, **Accepted Date:** May 26, 2024,
Published Date: May 31, 2024
Crossref doi: https://doi.org/10.37547/ajbspi/Volume04Issue05-10

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ABSTRACT

The process of formation of congenital anomalies occurs during certain time periods when organs are in a critical stage of development, known as organogenesis. At the stages of organogenesis, intensive formation and further development of organs occurs.

KEYWORDS

Organogenesis, dysmorphogenesis, colpopoiesis.

INTRODUCTION

The process of formation of congenital anomalies occurs during certain time periods when organs are in a critical stage of development, known as organogenesis. At the stages of organogenesis, intensive formation and further development of organs occurs. If damage or developmental delay occurs during these stages, it may be caused by exposure to substances known as teratogens. When the differentiation process is stopped, embryonic structures characteristic of a certain stage of organ development may be preserved (13, 23, 28). In recent years, there has been an increase in the detection of developmental defects of various organs and systems, including anomalies in the development of the genitals

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in girls (19). Failures in the development of female genital organs account for 4% to 14% of all congenital anomalies. Among them, the most socially significant anomaly is aplasia of the vagina and uterus, which occurs with a frequency of approximately 1 case per 4500-5000 live births of female children. (1,2,30,31). Aplasia of the vagina and uterus, also known as Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS), although not life-threatening, is a serious problem associated with psychological, social and reproductive difficulties. Modern advances in medicine make it possible to restore reproductive function in cases of complete infertility. However, the inability to engage in sexual activity is a source of significant emotional distress (1,4). So, aplasia of the uterus and vagina, also known as Rokitansky-Küster-Mayer syndrome, this condition is an extreme form of congenital dysmorphogenesis, which means underdevelopment of the organs of the reproductive system (26). Surgical treatment for aplasia of the uterus and vagina is aimed at creating an artificial vagina (colpopoiesis) in order to restore sexual function. The approach to surgical treatment in this case remains controversial: as a necessity only in the presence of functional activity or in pain (2,3,23). Genital prolapse, more often observed after reconstructive vaginal surgery for various reasons, is becoming a significant medical, social and economic problem, especially in the context of increasing life expectancy (18,20). According to WHO forecasts, by 2030 the number of women aged 80 years and over is expected to quadruple. This means that the problem of genital prolapse is becoming more pressing every year (8, 15,21). When women reach 80, one in five may require surgery for genital prolapse (32). In Russia, the incidence of genital prolapse varies from 15.0% to 40.0%. It accounts for approximately 15.0% of all gynecological interventions (18, 20,21). The peak incidence occurs at ages 50 years and older (14,32). Currently, there are approximately 300 methods of surgical treatment of genital prolapse, most of them based on minimally invasive approaches using various synthetic implants (10,11,17). Therefore, the incorrect choice of method for surgical treatment of genital prolapse and insufficient use of rational antibiotic therapy in the postoperative period may explain the high rate of recurrence of the disease (22). Research by Huser M. et al. (2012) and Togami J.M. et al. (2012) show that the recurrence rate when correcting genital prolapse using own tissue is 20.0–40.0%, while when using mesh implants this figure ranges from 3.0 to 20.0% (9.5 ,6,15). Genetically determined processes associated with the pathology of the synthesis of elastic fibers in the connective tissue of the pelvic floor structures were studied. The importance of the activity of matrix enzymes FBLN5, LOXL-1, MMP-2 and MMP-9 in various components of connective tissue, such as epithelium, vascular endothelium, extracellular matrix and fibroblasts, was revealed in various degrees of pelvic organ prolapse. In more complex cases of predicting the development of

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pelvic organ prolapse, genetic research can be supplemented by studying the activity of FBLN5 and MMP-9 in vaginal tissue, which complements genetic research in the diagnosis of preclinical forms of genital prolapse (12,25). The creation of an artificial vagina is a significant achievement in the field of plastic gynecology, providing women with optimal conditions and a natural opportunity for drainage of menstrual blood after surgery to create a neovagina. The primary material used for neovagina is the sigmoid colon, although other areas of the colon may occasionally be used (26,27). However, there is a lack of long-term clinical and morphological assessment with a complete study of adaptation processes over many years of functioning (16,17). This situation indicates a lack of specific data to study and evaluate the direction and characteristics of adaptive processes in the areas of the gastrointestinal tract used to create the neovagina (17,19). The issue of expanding and improving the optimal selection of segments for detubularization remains important, taking into account the individual characteristics of the patient and the long-term functioning of the artificial vagina. This highlights the need for a broad, comprehensive comparative analysis of clinical, endoscopic, morphological and immunohistochemical data (16). Histological examination of samples of a removed rudimentary uterus with complete aplasia of the cervix revealed severe dysplasia of the uterine tissue at the microscopic level: the uterine wall is represented by muscle tissue, which contains myocytes with pronounced signs of dystrophy, as well as areas of loose and rough connective tissue with areas of calcification. The endometrium covering the uterine cavity shows signs of atrophy. The cervical canal and cervix are absent. Histological examination of biopsies of a partially aplastic vagina also shows deep dysplastic changes at the cellular and tissue levels, which correlate with the level of vaginal aplasia. The degree of dysplastic lesions of the vaginal tissue is considered more pronounced with a higher level of aplasia.

(7). The main treatment method for this disease, including Rokitansky-Küster-Mayer syndrome, is the creation of an artificial vagina, or colpopoiesis. There are a variety of conservative and surgical methods for this purpose in the world. Vaginal dilatation, or colpoelongation, which is based on mechanical traction of the vulva's own tissues, is widely used as the first line of therapy in various countries. This method is characterized by the absence of surgical risk and demonstrates high clinical effectiveness (90-96%) (29). In connection with the above, there is no doubt about the need to search for methods of diagnosis, prognosis and increase the effectiveness of treatment in patients with aplasia of the uterus and vagina. In addition, studying the condition of the neovagina created from the sigmoid colon, assessing the structural changes in the transformation of single-layer epithelium into multilayer epithelium observed in it makes it possible

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to predict the survival rate of the neovagina of patients. Despite the large number and type of surgical interventions to create a neovagina, morphological changes, including cytological studies, have not been fully studied.

Purpose of the study: comparative cyto-morphological assessment of the colonic variant of vaginoplasty in the early and late stages of functioning, establishing, based on cytological studies, the biological features of the adaptation process with an individual prognostic assessment.

METHOD

The material was biopsy samples obtained after vaginoplasty in women who underwent surgery to create an artificial vagina. A comparative analysis of the results of cytological examination and treatment of 52 women was carried out. To study the morphological changes of the neovagina, samples for cytological and histological examination were taken from 34 patients with congenital anomalies of the vagina after surgery to create a neovagina. The average age was 24±7.5 years, in addition, a control group of 6 healthy individuals was selected. Inclusion criteria were: a confirmed diagnosis of Mayer-Rokitansky-Küster-Hauser syndrome and surgery for vaginal reconstruction; diagnosis and surgical treatment were performed depending on the anatomical form of the malformation, patient complaints, clinical manifestations and according to the ICD classification (Q51.8); a gentle method to prevent a risky procedure; early diagnosis of malignant processes; early diagnosis of neovaginal prolapse; associated with tissue biopsy; verification of tissue accommodation; early diagnosis of dysplastic and hyperplastic processes of the neovagina; Scientific research; After surgery to create a neovagina, all patients in the study groups were regularly examined by gynecologists and smears were taken for cytological examination of mucosal cells. Cytological examination of biopsy samples of the mucous membrane of the sigmoid colon was carried out from 1 to 10 years from the date of surgery. Classical cytology was performed. A special instrument (brush) was used to take a smear from the mucous membrane of the neovagina. A smear was applied with a brush onto dry glass, fixed, air dried and then stained. Manual staining of finished cytological preparations using a set of fixative and dyes "Leukodif 200". The patients' smears were processed and stained using the Romanovsky-Giemsa method and the Pappenheim method. To obtain statistically reliable results, at least 100 objects were analyzed in several fields of view. A morphometric study was carried out with the calculation of the following parameters: the number of neutrophil leukocytes, lymphocytes and epithelial cells was counted, after which the neutrophil-epithelial index (NEI) and lymphocyte-epithelial index (LEI), as well as the nuclear-cytoplasmic ratio of the surface cell layer (NSL) were calculated. Cytogram data were taken

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as the initial indicator. Statistical data processing. When studying micropreparations, cytological and cytomorphometric analyzes were performed. Cytomorphometric analysis was carried out using a scanner on a NanoZoomer microscope (REF C13140-21.S/N000198/HAMAMATSU PHOTONICS/431-3196JAPAN), using the NDP.VIEV2.0.,QuPath.0.4.0.url program.

RESULTS

The main groups of patients were divided into 3 subgroups:

1- patients with vaginal prolapse after surgery to create a neovagina after 1 year (12 patients);

2- patients with vaginal prolapse after surgery to create a neovagina after 3 years (14 patients);

3- patients with vaginal prolapse after surgery to create a neovagina up to 10 years of age (8 patients);

We performed a cytological study of the preparations in order to study the dynamics of the reparative processes of the neovaginal mucosa. The results of a cytological study showed that in patients with vaginal prolapse after surgery to create a neovagina after 1 year, the epithelium of the mucous membrane of patients aged 18-25 years was observed, indicating the presence of squamous epithelial cells and cellular accumulations of unchanged glandular epithelium. Using the cytological method, we also studied the dynamic change in the number of neutrophils, lymphocytic leukocytes and epithelial cells. The cytogram results showed that often in our observations in the group of women with neovagina there was a reduced number of superficial cells compared to intermediate and keratinizing cells. In the intermediate cells, slightly enlarged vesicular nuclei were found, which indicate esterogen deficiency. Along with them, cells of the upper rows of the superficial or intermediate layer can be found. In some cases, smears are represented by cells of the surface functional epithelium. In this observation group, patients partially produced esterogen (Fig.1). Such a picture of a smear is due to the body's good compensatory abilities.

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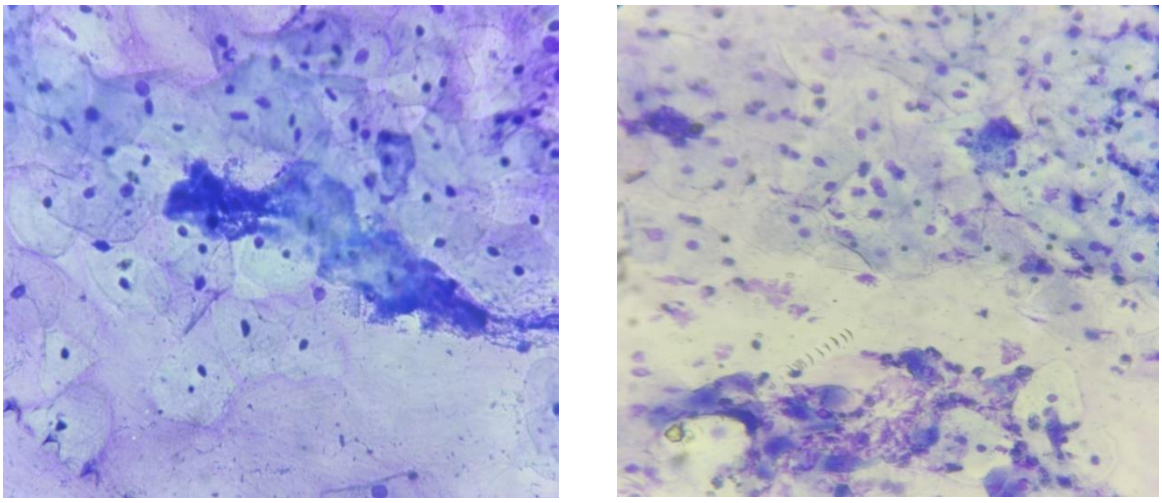

Fig.1. Cytogram of a patient in the observation group 1

year after surgery. Cells of squamous epithelium of the superficial and intermediate type. Poor microflora. The mucous membrane of the neovagina. Pappenheim staining, X40.

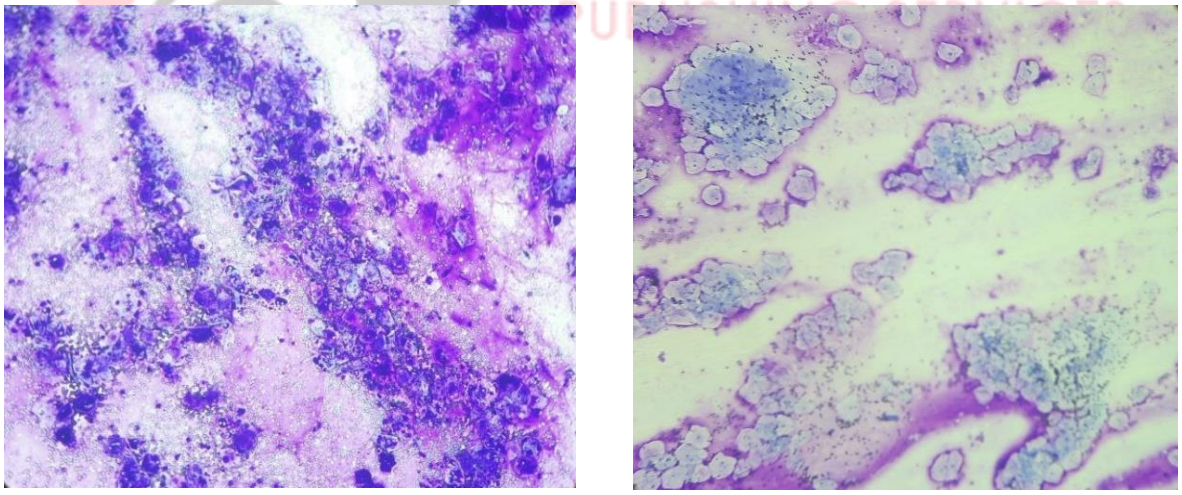

Fig.2. Cytogram of a patient in the observation group 1

year after surgery. Cells of squamous epithelium of the superficial and intermediate type. Degenerative changes in the cells of the surface layer. The mucous membrane of the neovagina. Pappenheim staining, X40.

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In summary, the data demonstrate that women with neovagina exhibit significant changes in the nuclear-cytoplasmic ratio in various mucosal cell types compared with controls. The stability of the NVC in the control group contrasts with the variability of indicators in the group of women with neovagina, which may indicate more pronounced adaptation and regenerative processes in the mucous membrane of the neovagina. In addition, in the observation group for the period of 3 and 10 years, clusters of intermediate and large parabasal cells were noted in the smears. All cells are well preserved, their nuclei are enlarged, equally large and poorly colored. The increase and accumulation of intermediate and parabasal cells demonstrates a pronounced estrogen deficiency (Fig.3.,4).

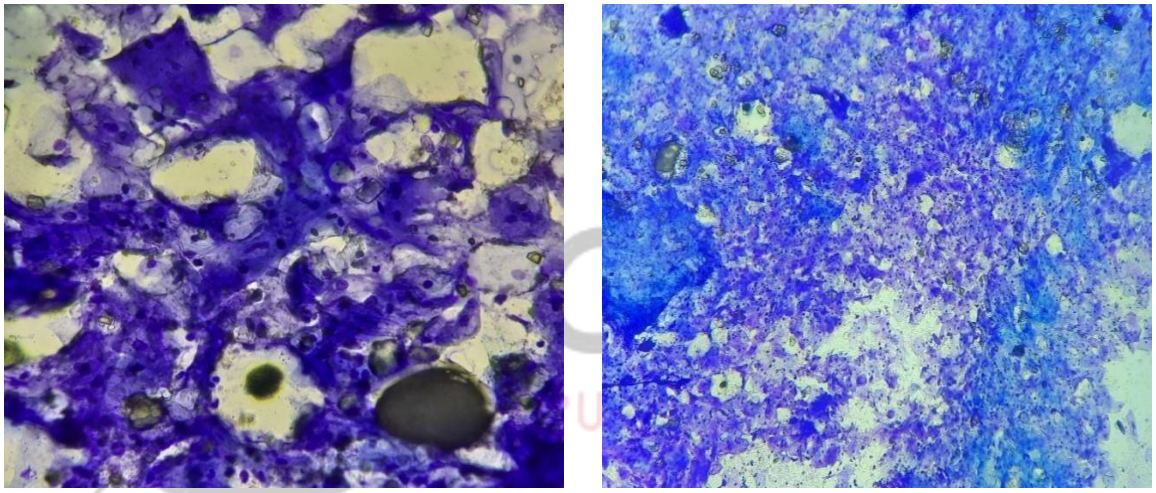

Fig.3. Cytogram of a patient in the observation group 4 years after surgery. Squamous epithelial cells of the superficial, intermediate and parabasal type. Destroyed cells of the parabasal layer in the form of "naked" nuclei. neovagina. Painting by Romanovsky-Gimse, X40.

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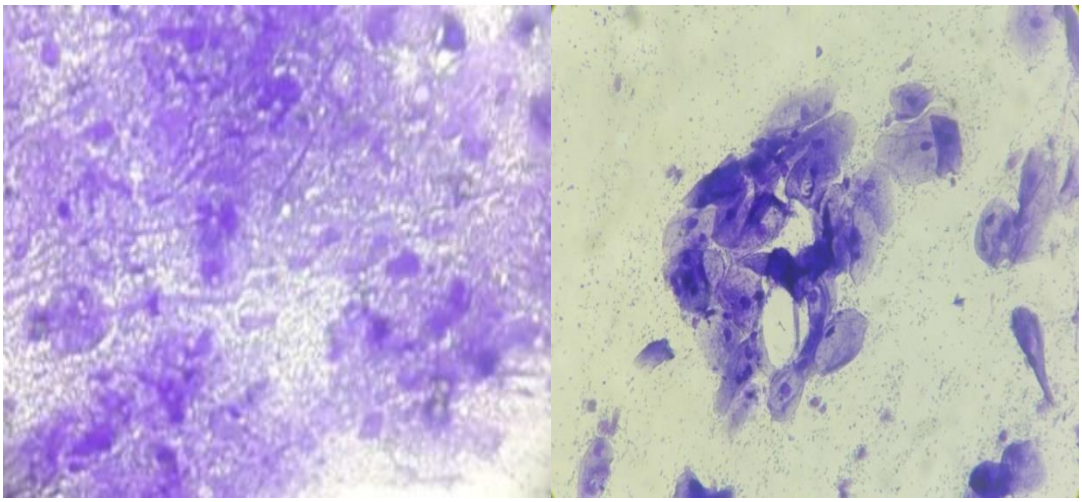

Fig.4. Cytogram of a patient in the follow-up group 6 years after surgery. The cells of the squamous epithelium are of a superficial, intermediate type. The surface cells are polygonal, fusiform, with karyorexis and karyopycnosis of the nuclei. Destroyed intermediate-type cells. neo vagina. Pappenheim coloring, X40.

So, cytological examination of smears in women with neovagina, especially in the periods 3 and 10 years after surgery, revealed significant changes indicating estrogen deficiency. Clusters of intermediate and parabasal cells with enlarged, weakly colored nuclei are observed. These changes are especially pronounced in women with aplasia of the uterus and appendages. Characteristic smears of the menopausal type, with the predominant presence of parabasal cells and signs of dystrophy, confirm insufficient hormonal stimulation and pronounced changes in the cellular composition of the neovaginal mucosa.

In addition, our observation groups included patients with impaired gender formation (Female and male false hermaphroditism). In the vaginal smears of these patients, mainly cells of the lower rows of the surface layer were found, with the presence of cells of the upper rows of the intermediate epithelium. In addition, the smears are represented relatively by cells with signs of atypia (Fig.5.,6).

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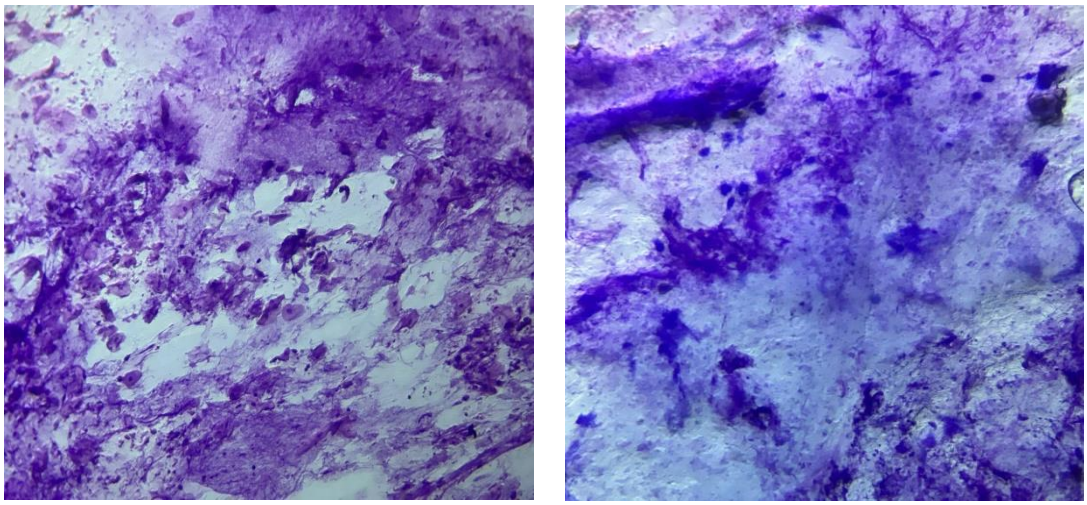

Fig.5. Cytogram of a patient in the follow-up group 10 years after surgery. The smear contains single cells of the squamous epithelium of the superficial, intermediate type. Surface cells, with karyorexis and karyopycnosis of the nuclei. Neovagina. Atrophic type of smear. Pappenheim coloring, X40.

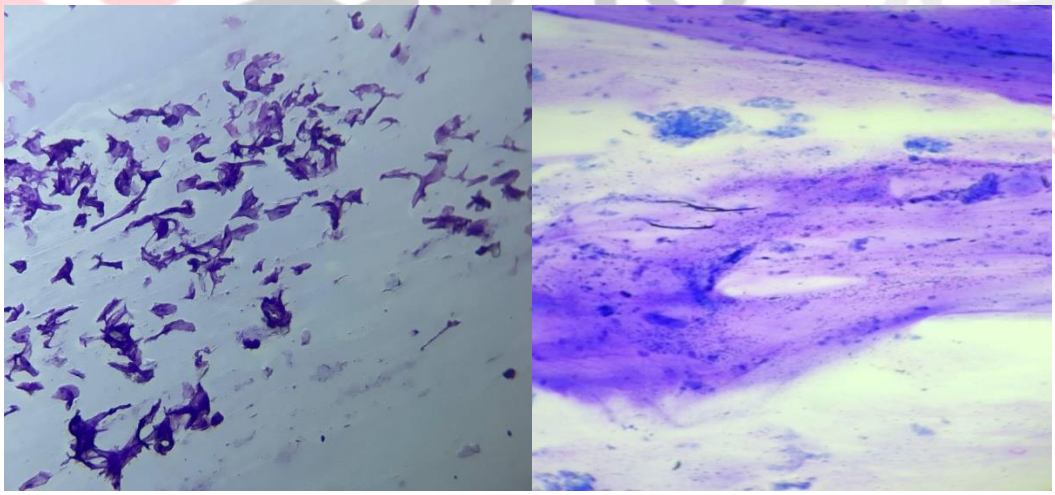

Fig.6. Cytogram of a patient in the follow-up group 10 years after surgery. The smear contains abundant squamous epithelial cells of the superficial and intermediate type. The surface cells are wrinkled, pyknotic, with karyorexis and karyopycnosis of the nuclei. Neovagina. Atrophic type of smear. Pappenheim coloring, X40.

Patients with impaired sex formation (female and male false hermaphroditism) showed specific changes in cytological smears. The main composition of cells is represented by cells of the lower rows of the surface

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layer and the upper rows of the intermediate epithelium.

The presence of cells with signs of atypia indicates possible pathological changes and requires further observation and analysis. These data emphasize the need for an individual approach in the diagnosis and treatment of patients with similar gender formation disorders. Since these patients lack ovarian function, they are partially compensated by adrenal harmonics, which enhance the release of androgen. Androgen acts on the vaginal epithelium and causes changes in epithelial cells, mainly by increasing the surface layer and intermediate type.

This smear of the patients is typical for the androgenic type of smear. Thus, estrogenic insufficiency causes the impossibility of complete maturation (differentiation) of the multilayer squamous epithelium. Due to the disappearance of the superficial – mature layers, the mucous membrane of the vagina is thinned, and the smear pattern is mainly represented by deep layers of the epithelium. Such changes in their smears proved a complete lack of estrogens and atrophy of the vaginal mucosa. And so, estrogen deficiency in patients with impaired sex formation leads to the impossibility of complete aging of the vaginal epithelium, which manifests itself in thinning of the mucous membrane and the predominance of deep layer cells in smears.

These changes indicate the complete absence of estrogens and atrophy of the vaginal mucosa, confirming the need for careful hormonal control and an individual approach in the treatment of this group of patients.

Table 1 shows changes in the nuclear-cytoplasmic ratio (NCC) in various types of cells of the flat non-corneating epithelium of the neovagina in comparison with the control group over three periods: up to 1 year, up to 3 years and up to 10 years after surgery.

Table No. 1.

Dynamic changes in the nuclear-cytoplasmic ratio in smears-prints of the neovagina mucosa (microns)

Observation period	Types of cells of the flat non-corneating epithelium neovagines	Nuclear-cytoplasmic ratio of cells in the control group (n=20)	Nuclear-cytoplasmic ratio of neovaginal mucosal cells in women of the observation group (n=34)
Up to 1 year	Surface cells	50:5 microns (1:10)	33:5 microns (1:6)
	Intermediate cells	30:6 microns (1:5)	24:6 microns (1:4)
	Basal cells	15:5 microns	25:5 microns

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		(1:3)	(1:5)
Up to 3 years old	Surface cells	50:5 microns (1:10)	47:7 microns (1:6)
	Intermediate cells	30:6 microns (1:5)	56:6 microns (1:9)
	Basal cells	15:5 microns (1:3)	48:6 microns (1:8)
Up to 10 years old	Surface cells	50:5 microns (1:10)	74:7 microns (1:10)
	Intermediate cells	30:6 microns (1:5)	67:8 microns (1:8)
	Basal cells	15:5 microns (1:3)	56:9 microns (1:6)

The surface cells of the mucous membrane, which is covered with a multilayer flat non-corneating epithelium, in women in the control group from 1 year to 10 years of follow-up, the NCC is 1:10 (50:5 microns). In women with neovagina, at 1 year 1:6 (33:5 microns), at 3 years of follow-up 1:6 (47:7 microns) and at 10 years of follow-up, the JCC gradually increases to 1:10 (74:7 microns). In intermediate cells, there are also changes in the JCC in women with neovagina, these indicators show a gradual increase in them. If for 1 year they were 1:4 (24:6 microns), for 3 years 1:9 (56:6 microns), then for 10 years of follow-up the indicators increased markedly by 1:8 (67:8 microns).

At the same time, the indicators of the JCC in the control group are 1:5 (30:6 microns).

The JCC of the basal cells of the multilayer squamous epithelium of the mucous membrane of the neovagina radically change their indicators from 1 year of observation 1:5 (25:5 microns), to 3 years 1:8 (48:6 microns) and after 10 years, 1:6 (56:9 microns). In the control group, the JCC is 1:3 (15:5 microns).

Thus, the JCC of surface cells in the control group remains stable at the level of 1:10 throughout the entire period, whereas in the comparison group with neovagine, fluctuations from 1:6 to 1:10 are observed.

The JCC of intermediate cells is stable at level 1:5. In neovagin, it changes from 1:4 to 1:9. The number of basal cells is constant (1:3). In neovagin, it varies from 1:5 to 1:8. These data demonstrate changes in the nuclear-cytoplasmic ratio in various types of cells of the neovagin mucosa compared with the control group.

The results of our observations showed that in patients with vaginal prolapse after surgery to create a neovagina after 1 year, cylindrical epithelial cells,

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parabasal, basal cells were found in vaginal smears. They had obvious inflammatory cell infiltrates. Infiltrates mainly consisted of lymphocytes, neutrophils and fibroblasts.

In addition, in the smears of patients with vaginal prolapse after surgery to create a neovagina after 3 years, mainly surface cells in a state of dystrophy of the cells of the cylindrical epithelium were found.

Basal cells were subjected to hyperplasia and hyperchromia. Parabasal cells were located separately. The smears were dominated by lymphocytic cells rather than neutrophilic leukocytes and fibroblasts.

In the smears of patients with vaginal prolapse after surgery to create a neovagina up to 10 years old, cells of the intermediate epithelium were usually found, separate cells of the parabasal layer and the basal layer appeared in a state of hyperplasia and dysplasia (Fig.7).

In them, the nuclei are hyperchromic, in some epithelial cells with doubled nuclei. Lymphocytes also predominated in the smears of patients in this observation group, rather than neutrophilic leukocytes and fibroblasts (Fig.8.,9).

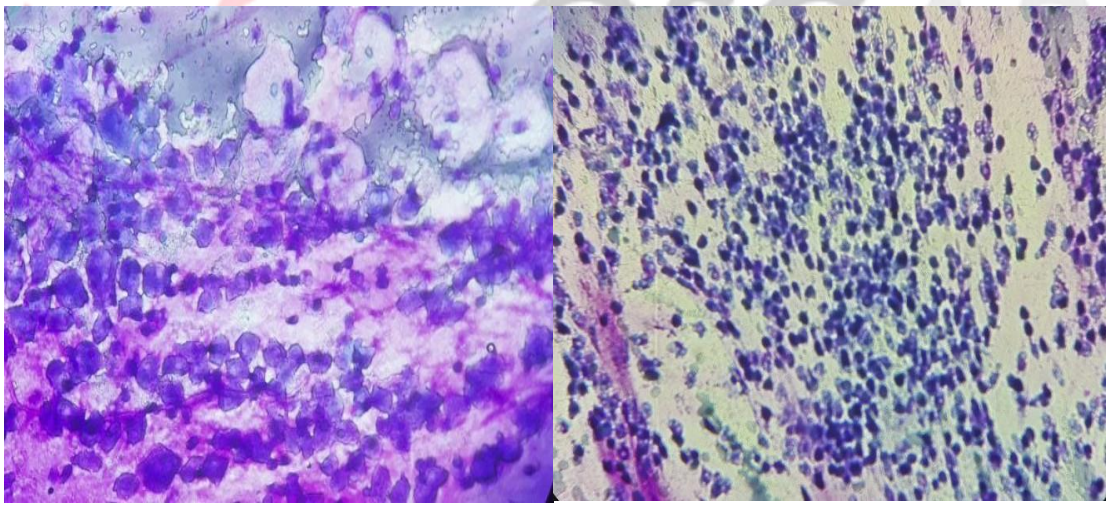

Fig.7. Cytogram of a patient in the follow-up group 1 year after surgery. Degenerative changes in the cells of the cylindrical epithelium in the mucus strands. Single surface cells with karyorexis and karyopycnosis of the nuclei. Pronounced lymphocytic infiltration of the mucous membrane of the neovagina. Pappenheim coloring, X40.

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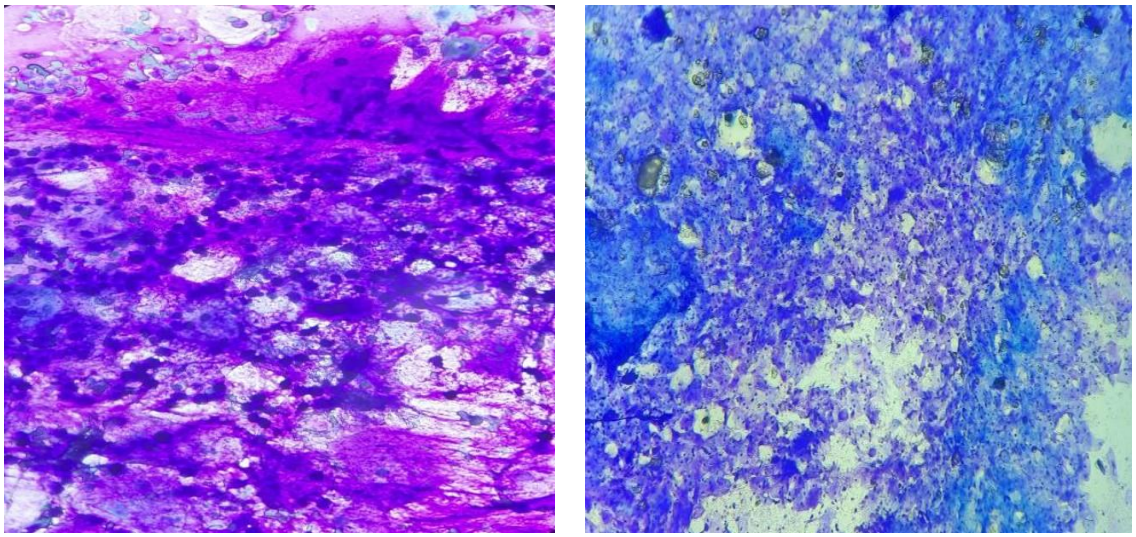

Fig.8. Cytogram of a patient in the observation group 3 years after surgery. In smear b, there is pronounced lymphocytic infiltration, surface and intermediate cells in a state of dystrophy and hyperkeratosis. Neovagina. Painting by Romanovsky-Gimse, X40.

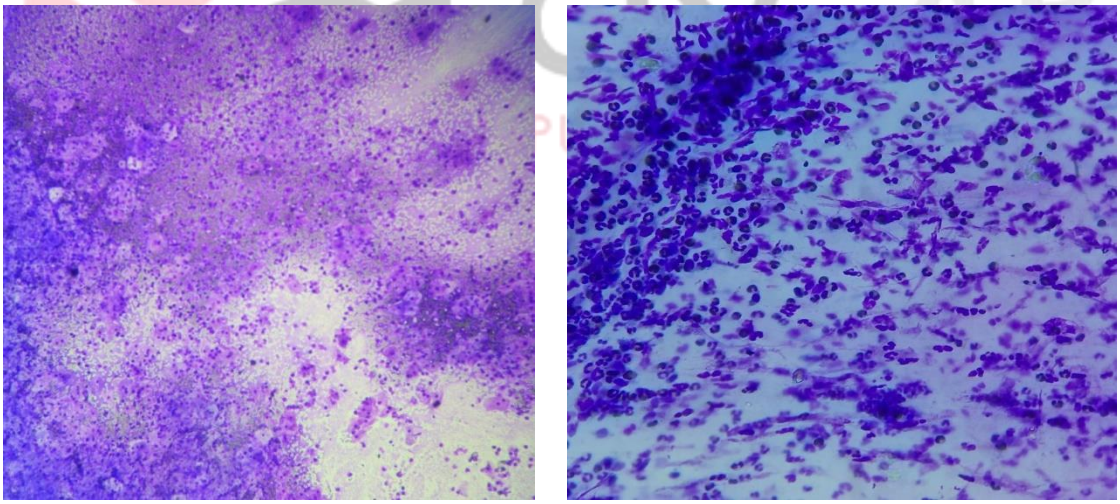

Fig.9. Cytogram of a patient in the follow-up group 8 years after surgery. Single surface cells with degenerative changes. Stroma reaction (neutrophils and multinucleated histiocytes). Pappenheim coloring, X40.

So, in patients with vaginal prolapse, after surgery to create a neovagina, significant changes in the cellular composition in the smears are observed. Inflammatory infiltrates predominate for 1 year, after 3 years the number of dystrophic and hyperplastic cells increases, and by the age of 10 signs of dysplasia appear.

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Lymphocytes predominate throughout the observation period, indicating a chronic inflammatory process. These data emphasize the need for long-term follow-up and treatment adjustments to prevent complications and maintain the health of the neovaginal mucosa.

In addition, dynamic changes in the nuclear-cytoplasmic ratio in smears-prints of the mucous membrane of the neovagina were studied. From Table No. 1. it can be seen that the quantitative indicators of neutrophils, lymphocytes and epithelial cells differ compared to the control group. The cellular composition of the control group of neutrophilic leukocytes ranged from 15.45% to 17.55%, with a return to 15.65% after 10 years. The indicators of postoperative neovagina show a gradual decrease from 94.70% to 68.45% after 10 years.

If the lymphocyte counts of the control group remain stable at about 16% throughout the entire period, then in the group of women with neovagina they range from 95.45% to 85.50% after 10 years.

In the control group of observation, epithelial cell counts gradually increase with the age of women and the period of observation, they have an increase from 67.80% to 85.48% after 10 years.

Whereas in women with neovagina, the number of epithelial cells was reduced by 1 year of follow-up (34.52%) and gradually increased by 3 and 10 years from 34.52% to 54.73%. This cytological picture corresponds to the degenerative-inflammatory period after the surgical process.

Table No.1.

Dynamic changes in quantitative indicators of neutrophilic leukocytes (%) in smears of the neovaginal mucosa

Observation period	The number of neutrophils in the control group (n=18)	The number of neutrophilic leukocytes, (%)
Up to 1 year	15,45±0,32	94,70±0,76
Up to 3 years old	17,55±1,08	87,65±0,38
Up to 10 years old	15,65±0,32	68,45±3,43
Observation period	**The number of lymphocytes in the control group (n=18)**	**The number of lymphocytes in women with neovagina, (%)**
Up to 1 year	16,05±0,64	95,45:0,32
Up to 3 years old	15,95±1,51	90,55±1,08

American Journal Of Biomedical Science & Pharmaceutical Innovation
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OCLC – 1121105677
Publisher: Oscar Publishing Services

Up to 10 years old	15,97±1,51	85,50±0,32
Observation period	**The number of epithelial cells in the control group (n=18)**	**Number of epithelial cells, (%)**
Up to 1 year	67,80±0,42	34,52±1,50
Up to 3 years old	73,65±1,06	46,67±1,30
Up to 10 years old	85,48±0,62	54,73±1,70

DISCUSSION OF THE RESULTS

The results of the cytogram showed the following features of the cellular composition in women with neovagina, there is often a decrease in the number of surface cells compared with intermediate and keratinizing cells. Enlarged vesicular nuclei were found in intermediate cells, which may indicate a lack of estrogens. There may be a variety of cells in the smears. In some cases, smears are represented by cells of the superficial functional epithelium. In patients who partially produce estrogen, which is reflected in the presence of cells characteristic of the superficial functional epithelium. Such a cytogram is due to the good compensatory abilities of the body, which partially compensates for the lack of estrogens. These cytograms demonstrate that women with neovagina have a decrease in the number of surface cells, an increase in vesicular nuclei in intermediate cells and the presence of cells of the upper layers of the epithelium. These changes indicate insufficient estrogenic stimulation, but also indicate significant compensatory abilities of the body to maintain partial estrogenic activity.

Data analysis shows that the nuclear-cytoplasmic ratio (NCC) of surface cells in the control group remains stable at 1:10 throughout the entire follow-up period, while fluctuations from 1:6 to 1:10 are observed in the group of women with neovagina.

The JCC of intermediate cells also remains stable at the level of 1:5 in the control group, but in women with neovagina it varies from 1:4 to 1:9. The number of basal cells in the control group is constantly 1:3, whereas in women with neovagina it varies from 1:5 to 1:8.

CONCLUSION

It can be concluded that women with neovagina have significant changes in the cellular composition of the mucous membrane compared with the control group.

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Neutrophilic leukocytes and lymphocytes decrease more markedly in women with neovagina, whereas epithelial cells show an increase over time, but remain below the values of the control group throughout the entire follow-up period.

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