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Treatment of the Mayer-Rokitansky-Küster-Hauser syndrome with autologous in vitro cultured vaginal tissue: descriptive study of long-term results and patient outcomes.

Running title: Vaginoplasty for the treatment of Rokitansky syndrome

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ABSTRACT

Objective: Evaluating sexual function and quality of life (QoL) in patients treated with a modified Abbé-McIndoe technique using in vitro cultured autologous vaginal mucosa.

Design: Descriptive study.

Setting: Policlinico Umberto I, “Sapienza” University of Rome.

Population: From 2006 to 2016, 39 women affected by Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS) underwent vaginoplasty at our center using a modified Abbé-McIndoe technique with in vitro cultured autologous vaginal tissue.

Methods: For each patient, vaginal tissue was obtained by full-thickness biopsy of the vaginal vestibule. Following enzymatic dissociation, cells were cultured for 2-3 weeks before the transplant.

Main outcome measures: Each patient completed two validated questionnaires to quantify sexual function and QoL: the Female Sexual Function Index (FSFI), administered at 12, 36 and 60 months, and the Psychological General Well Being Index (PGWBI) administered at 0, 6 and 36 months after surgery.

Results: Twelve months after surgery, 29 patients have engaged in regular sexual activity. The FSFI test results show a satisfactory sexual function compared to general population, with a median at 12 months of 25.85 (range 4.6-30.5), 36 months of 27.2 (range 4.4-33.6) and 60 months of 29.6 (range 23.9-33.6). The PGWBI

questionnaire shows a median score of 420.5 (range 108-540) before surgery, and 459 (range 252-533) at the last follow-up.

Conclusions: Vaginoplasty performed with the use of autologous vaginal tissue, besides ensuring a long-term satisfying sex life, helps in achieving an improvement in QoL that is maintained over time.

Funding statement: No external financing was obtained to conduct the study

Key words: Mayer-Rokitansky-Küster-Hauser syndrome, vaginoplasty, autologous tissue, quality of life, sexual function.

Tweetable abstract: Vaginoplasty using in vitro vaginal tissue ensure a satisfactory sexual function and improves quality of life.

INTRODUCTION

The Mayer-Rokitansky-Küster-Hauser Syndrome (MRKHS) is the most common form of vaginal agenesis and the second most common cause of primary amenorrhea; the incidence has been estimated as 1 in 4,500 female births [ACOG, 2002; Burel *et al*, 2006; Karine *et al*, 2007; Devriendt *et al*, 1999]. This syndrome is subdivided in two types: type I (isolated) or Rokitansky sequence (OMIM 277000), in which only the caudal part of the Müller duct results altered, and type II or Müllerian duct aplasia, Renal dysplasia and Cervical Somite anomalies (MURCS) association (OMIM 601076), often associated with other malformations involving the upper urinary tract, the skeleton and the otologic sphere; heart malformations are more rarely reported [Schmid-Tannwald, Hauser, 1977; Duncan *et al*, 1979; Benedetti Panici *et al*, 2007]. Therefore, a correct diagnosis of Müllerian agenesis includes

investigations to identify the main features, to help frame the typical defects and any associated malformations [Reindollar *et al*, 1981].

The primary end-point of the treatment of vaginal agenesis is to allow women to have a satisfactory sexual life, accordingly the ideal treatment should aim to achieve an anatomically and functionally appropriate neovagina. The goal of any method is to create a vaginal canal of adequate diameter and length and appropriate axis to accommodate sexual intercourse. It should also have appropriate location, postero-superior direction, suitable size, hairless walls and it should be coated with elastic tissue and a layer of mucosa. In addition, the vagina should have a normal amount of secretion and lubrication and should require minimal care for maintenance [Soong *et al*, 1996]. The literature describes various methods for the treatment of MRKHS, each one with advantages and disadvantages. In 2006, our group developed a new technique for the treatment of Müllerian agenesis modifying the Abbé-McIndoe surgical procedure using in vitro cultivated autologous vaginal tissue to cover the walls of a neovagina surgically created in the space between rectum and bladder [Benedetti Panici *et al*, 2007].

The intervention of vaginoplasty is an opportunity to achieve a normal sex life, and this has a major impact on Quality of Life (QoL). The most important parameter to consider in order to confirm the anatomical success of the intervention during the follow-up period is patient sexual satisfaction, which is directly proportional to the treatment success.

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The aim of this study is the evaluation of the long-term sexual function and QoL in patients treated with the modified Abbè–McIndoe technique, with the use of in vitro cultured autologous vaginal mucosa.

PATIENTS AND METHODS

Between 2006 and 2016, 39 Caucasian women with MRKH syndrome were referred to our institution. All patients exhibited primary amenorrhea, normal secondary sexual characteristics, and a vaginal dimple without vaginal orifice. All women underwent a pre-operative work-up, which included trans-abdominal ultrasound scan, chromosome analysis, abdominopelvic MRI, ECG, echocardiogram and chest X-ray. It was crucial to assess the patient's motivation and desire to undergo the operation as the success of the procedure is greatly influenced in the post-surgery phase by the patient continue to use the dilators needed to ensure the patency of the neovagina. The patients selected for surgery are those who expressed the need to begin to lead a normal sexual life and express their womanhood in a complete manner.

Patients who could be treated by the Frank conservative method, patients with uncertain diagnosis of MRKHS and patients without suitable and sufficient vaginal tissue were excluded. Similarly, the surgery is not recommended in cases of alterations in coagulation processes and tissue repair.

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Each patient with MRKHS was subjected to a full-thickness mucosal biopsy from the vaginal vestibule. After enzymatic dissociation, cells were inoculated onto collagen IV (10 mg/dL) coated plates and cultured for 2 to 3 weeks. (Appendix S1). The patients were subjected to vaginoplasty with a modified Abbè-McIndoe technique with autologous in vitro cultured vaginal tissue, as previously reported [Benedetti Panici *et al*, 2015]. Patients underwent clinical follow-up visits at 1, 3, 6, and 12 months after surgery and every year thereafter. Clinical follow-up included vaginal examination and vaginostomy. Both vaginal cytology and vaginal biopsy were obtained at the 3-month follow-up visit.

At 6 months, this procedure can be declared a success if the neovagina has not undergone any shrinkage, does not give off unpleasant smells, does not cause vaginal dryness and presents a normal vaginal flora [Benedetti Panici *et al*, 2015].

To quantify QoL and sexual satisfaction, two questionnaires, both validated and in the Italian language, the Female Sexual Function Index (FSFI) and the Psychological General Well Being Index (PGWBI), were administered to each patient.

Functional results were assessed using the FSFI questionnaire at 12, 36 and 60 months after surgery. The FSFI, a 19-item questionnaire, has been developed as a brief, multidimensional self-reported instrument for assessing sexual function in women. Based on factor analytic methods, six factors or domains of sexual function were identified: (a) desire (b) subjective arousal, (c) lubrication, (d) orgasm, (e) satisfaction, and (f) pain/discomfort. A scoring system was developed for obtaining

individual domain scores and a total score. A total score of 26.55 is the cut-off proposed to assess sexual function as lower values indicate an impairment in sexual function. The highest total FSFI score that may be obtained is 36. The mean score in the normal population is 30, so a good functional result can be considered when the total score ranges between 30 and 36, satisfactory between 23 and 29, and poor 23 or less [Rosen *et al*, 2000].

Before the intervention and then 6 and 36 months after surgery a questionnaire for the assessment of QoL was administered to each patient. The choice of this questionnaire is related to the extreme strength of the questionnaire, its high reproducibility and internal consistency and meticulous methodological framework that supports it [Dupuy *et al*, 1984]. The original PGWBI consists of 22 self-administered items, rated on a 6-point scale, which assess psychological and general well-being of respondents in six Health-Related QoL domains: anxiety, depressed mood, positive well-being, self-control, general health and vitality. Each item is rated with a numerical value (ranging from 100: perfect health, to 0: worst standard of health). The total scores derived from each domain give an overall score, which expresses the degree of psychological and emotional well-being and which is correlated with the general population of the same sex and similar age (median standardized profile).

No external financing was obtained to conduct the study.

RESULTS

The operation was performed successfully in all 39 patients. Patients characteristics are presented in **Table 1**. The mean age of patients at surgery was 18.5 years-old (range 16-49 years-old). Twenty-two patients (57%) were affected by MRKHS type I, 17 patients (43%) had type II. Fourteen patients (36%) had two non-functioning rudimentary emi-uteri, 12 patients (32%) had complete agenesis of the uterus and the fallopian tubes, 10 (25%) patients had a single hypoplastic uterus and 3 (7%) a functioning hypoplastic uterus, causing cyclical pelvic pain, who were treated with estrogen-progestin therapy. Renal abnormalities were present in 10 patients (25%), congenital heart defect in 3 patients (8%), skeletal malformations in 3 patients (8%), and 4 patients (10%) had abnormal hearing. All patients tolerated the procedure well and there were no intraoperative complications.

The surgical data are presented in **Table 2**. The size of the vaginal biopsy ranged from 0.5 to 2 cm² (mean 1 cm²). The period between the biopsy and the development of the fully differentiated mucosal tissue ranged from 15 to 36 days (mean time 21 days). The mean operative time was 23 minutes (range 15-60 min) and mean blood loss was 85 ml (range 20-110 ml). Post-operatively, all the patients were placed on a liquid diet until day 3 and a Foley bladder catheter was maintained to avoid contamination of the surgical site by urine and feces. After the first surgical medication, the Foley was removed and all patients were instructed to perform bladder training. No postoperative complications were reported. The length of

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hospitalization ranged from 2 to 16 days. The vaginal tissue percentage of attachment, pointed out during the first post-operative examination of the neovagina, was satisfactory and it was between 50% and 100% (mean percentage 80%). Patients started using the dilators autonomously after a mean post-operative time of 11 days (range 3-16 days).

As anatomical result, the median length of the vaginal canal was 8 cm (range 6-12) with a width of 2 cm. These dimensions were found unchanged at follow-up of 12 months. Cytology and biopsy histology performed at 3 months showed a normal vaginal mucosa without signs of atrophy.

One year after surgery 29 patients resumed regular sexual activity. They completed the FSFI questionnaire and the results were encouraging, obtaining a median score of 25.85 (range 4.6-30.5). The second follow-up was performed for 21 patients and the FSFI questionnaire showed a median score of 27.2 (range 4.4-33.6). The third administration of the FSFI questionnaire confirmed the expected results, with a median score of 29.6 (range 23.9-33.6) for 18 patients. Before surgical treatment, patients also completed the PGWBI questionnaire, which showed a median total score of 420.5 (108-540 score). The same questionnaire was also completed at the last follow-up, showing an increasing in median total score, amounting to 459 (range 252-533). At the time of data cutoff, 18 patients reached the 60 months follow-up for the third administration of the FSFI questionnaire and 15 patients reached the third administration of the PGWBI questionnaire. There were no loss to follow-up

patients. Patients underwent surgery at different times and the administration of the questionnaires was performed at the relevant follow-up times for each patient. FSFI and PGWBI questionnaires results are showed on Table 3.

DISCUSSION

Main findings:

In the last century, various therapeutic approaches have been proposed to treat vaginal agenesis, each one with advantages and disadvantages. From the first description of Abbé [Abbé 1898], many changes have been introduced to the technique, the most significant ones concerning the lining material of the newly formed canal. In our experience with the modified Abbé-McIndoe's surgery, using autologous in vitro cultured vaginal tissue, the graft is autologous and orthotopic, without any risk of rejection from the recipient site. The procedure is fast and does not leave scars. The major disadvantage of this technique is the cellular therapy, which means that it can only be performed in specialized centers with a cell factory which meets the Good Manufacturing Practice (GMP) requirements, and that it is necessary to coordinate the availability of the operating room with the needs of the laboratory when the tissue is ready to be transplanted.

Strength and limitations:

Since 2006, there has been a refinement of our surgical technique. The operative time and intraoperative blood loss decreased. We also improved the post-operative management, shortening the length of stay, making the patients self-sustaining at home in less time with regard to the use of vaginal dilators. The use of vaginal mucosa grown in vitro is still a very new technique, but our reported data show the use of autologous tissue grown in vitro is feasible and safe for patients affected by the MRKHS. The re-epithelialization is rapid because of the autologous material and there is a chance to get enough tissue to cover the whole vaginal canal. In addition, after the removal of gauzes and vaginal dilator, which is kept in place to apply further pressure of the newly formed vaginal tissue to the vaginal wall, the epithelialization may be noted for the entire length of the vaginal walls. This suggests that the epithelialization comes from the transplanted tissue and not, as in other techniques, from the vaginal vestibule tissue. Additionally, a recently published study, extensively describes the characteristic of vaginal mucous grown in vitro, proving the epithelial characteristics and the existence of mucinous glands between the grown cells, that give a normal glandular function to the new vagina. Furthermore, a patient's idea of having a new vagina consisting of their "own" vaginal tissue plays a significant role, psychologically and emotionally, in order to have a normal sex life [Benedetti Panici *et al*, 2015; Nodale *et al*, 2014].

Interpretation:

The main objective of this procedure is assuring good sexual function. Our results, obtained through the administration of the FSFI, show a satisfactory sexual activity, which maintains and improves over time. Furthermore, this technique has a good impact on the patient's QoL, with a higher score compared to the admission psychological-emotional state using PGWBI, and in terms of the median standardized profile of the general population of the same sex and same age. Moreover, the awareness of a patient that the neovagina consists of vaginal tissue, plays a very important role with regard to the improved psychological-emotional status to have a regular sexual life, which remains the first true endpoint of vaginal agenesis treatment. Our results suggest that the intervention of vaginoplasty with autologous vaginal tissue improves QoL, beside ensuring a satisfying sex life.

CONCLUSION

In conclusion, we have shown that vaginal autologous tissue grown in vitro is the ideal material for vaginoplasty in patients with MRKHS. This technique is innovative, the treated patients stop being "different", "sick" and "lacking something", and develop a positive self-perception that persists over time.

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Contribution to authorship: All the authors meet the criteria for authorship and gave their approval to the final version of the manuscript.

Authors contribution:

-Dr I. S.: performed the experiment, analyzed the data and wrote the paper;

-Dr I. P.: conceived and design the experiment, performed the experiment, analyzed the data;

-Dr C. M.: conceived and design the experiment, contributed reagents/materials/analysis tools;

-Dr L. M.: conceived and design the experiment and performed the experiment;

-Dr C. D. M.: analyzed the data and wrote the paper;

-Dr M. G.: performed the experiment, analyzed the data and wrote the paper;

-Dr A. M.: performed the experiment and analyzed the data;

-Dr S. C.: contributed reagents/ materials/ analysis tools;

-Dr E. V.: contributed reagents/ materials/ analysis tools;

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-Dr P. B. P.: conceived and design the experiment, performed the experiment,
analyzed the data;

Ethics approval

Name of the ethics committee: "Comitato etico dell'università "Sapienza".

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REFERENCES

1. ACOG: Committee Opinion. Number 274, July 2002. Non surgical diagnosis and management of vaginal agenesis. *Obstet Gynecol* 2002, 100:213-216
2. Burel A., Mouchel T., Odent S., Tiker F., Knebelmann B., Pellerin I., *et al.* Role of HOXA7 to HOXA13 and PBX1 genes in various forms of MRKH syndrome (congenital absence of uterus and vagina). *J Negat Results Biomedd* 2006, 5:4
3. Morcel K., Watrin T., Pasquier L., Rochard L., Le Caignec C., Dubourg C., *et al.* Mayer-Rokitansky-Küster-Hauser Syndrome. *Orphanet Journal of Rare Disease* 2007, 2:13
4. Devriendt K., Jaeken J., Matthijs G., Van Esch H., Debeer P., Gewillig M., *et al.* Haploinsufficiency of the HOXA gene cluster, in a patient with hand-foot-genital syndrome, velopharyngeal insufficiency, and persistent patent Ductus botalli. 1999 Jul; 65 (1): 249-251
5. Schmid-Tannwald I, Hauser GA. Atypical form of Mayer-Rokitansky-Küster-Hauser Syndrome. *Geburtshilfe Frauenheilkd* 1977, 37:386-392
6. Duncan PA., Shapiro LR., Stangel JJ., Klein RM., Addonizio JC. The MURCS association: Müllerian duct aplasia, renal aplasia and cervicothoracic somite dysplasia. *J Pediatric* 1979, 95:399-402
7. Benedetti Panici P., Bellati F., Boni Terenzio., Francescangeli F., Frati L., Marchese C. Vaginoplasty using autologous in vitro cultured vaginal tissue in a patient with Mayer-Von-Rokitansky-Küster-Hauser syndrome. *Human Reproduction*, pp1-4, 2007
8. Reindollar RH, McDonough PG. Delayed sexual development: a study of 252 patients. *Am J Obstet Gynecol* 1981, 140:371-380
9. Soong YK., Chang FH., Lai YM., Lee CL, Chou HH. Results of modified laparoscopically assisted neovaginoplasty in 18 patients with congenital absence of vagina. *Human Repr* 1996, 11:200-203

10. Benedetti Panici P., Maffucci D., Ceccarelli S., Vescarelli E., Perniola G., Muzii L., *et al.* Autologous in vitro cultured vaginal tissue for vaginoplasty in women with Mayer-Rokitansky-Küster-Hauser syndrome: anatomic and functional results. *J Minim Invasive Gynecol.* 2015 Feb;22(2):205-11
11. Rosen R., Brown C., Heiman J., Leiblum S., Meston C., Shabsigh R., *et al.* The Female Sexual Function Index (FSFI): A multidimensional self-report instrument for the assessment of female sexual function. *J Sex Marital Ther* 2000;26:191-208
12. Dupuy HJ. The Psychological General Well-Being Index. 1984
13. Abbè R. New method of creating a vagina in a case of congenital absence, *Med Rec* , 1898, vol. 54 (pg. 836-838)
14. Nodale C, Vescarelli E, D'Amici S., Maffucci D., Ceccarelli S., Monti M., *et al.* Characterization of human vaginal mucosa cells for autologous in vitro cultured vaginal tissue transplantation in patients with MRKH syndrome. *BioMed Res Int.* 2014;2014:201518

Table legends:

Table 1. Patients characteristics

Table 2. Surgical data

Table 3. FSFI and PGWBI questionnaires results

TABLE 1. Patients characteristics

Mean age 18.5 (range 16-49)	
	N. of patients (%)
MRKHS	
Type I	22 (57)
Type II	17 (43)
Uterine anomalies	
2 rudimentary emiuteri	14 (36)
Agenesis	12 (32)
Hypoplastic	10 (25)
Hypoplastic functioning	3 (7)
Renal anomalies	
Absent	29 (75)
Present	10 (25)
Others	
Congenital heart defect	3 (8)
Skeletal malformations	3 (8)
Hearing problems	4 (10)

TABLE 2. Surgical data

	Mean (range)
Operative time (<i>min</i>)	23 (15-60)
Blood loss (<i>ml</i>)	85 (20-110)
Biopsy (<i>cm</i>)	1 (0,5-2)
The development of the fully differentiated mucosal tissue (<i>days</i>)	21 (15-36)
Hospitalization stay (<i>days</i>)	11 (3-16)
Percentage of take (%)	80 (50-100)
Day first medication (<i>days</i>)	7 (3-7)

TABLE 3. FSFI and PGWBI questionnaire results

Follow up (<i>months</i>) (n. of patients)	FSFI median score (range)
12 (29)	25,85 (4,6-30,5)
36 (21)	27,2 (5,4-33,6)
60 (18)	29,6 (23,9-33,6)
Follow up (<i>months</i>) (n. of patients)	PGWBI median score (range)
0 (39)	420,5 (108-540)
6 (17)	436 (108-502)
36 (15)	459 (252-533)