Vaginal reconstruction with two lower abdominal skin flaps in rabbits: Histological and macroscopic evaluation

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Vaginal reconstruction with two lower abdominal skin flaps: histological and macroscopic evaluation

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ABSTRACT

Objective: To evaluate the use of two skin flaps of the lower abdominal wall in the creation of a cylindrical conduit in vaginal reconstruction surgery in rabbits, through macroscopic and histological analysis.

Study design: An experimental study was performed in 16 female New Zealand rabbits, consisting of the use of two rectangular-shaped skin flaps of the lower abdominal wall measuring 1 cm longitudinally and 3 cm transversely anastomosed to each other through continuous suture of the edges of the two flaps to create a tube. Hysterectomy and excision of the vaginal vault were performed, and the skin tube was anastomosed to the remaining vaginal stump with separate points of polyglycolic acid 4.0. Animals were divided into 4 groups according to the euthanasia at 2, 4, 8 and 12 weeks, when after excision of the neovagina, macroscopic and histological evaluation with hematoxylin–eosin and Masson trichrome were performed.

Results: Of 16 operated rabbits, only 1 presented partial abdominal wall dehiscence, not compromising the flap viability. The macroscopic analysis of the vaginal conduit showed that it was kept open throughout the experimental steps, with a good patency and gauge, showing a slight retraction in the skin conduit length of no statistical significance. In the histopathological analysis, a local inflammatory process in the anastomosis was observed, which was larger in the early evaluation but decreased in late evaluations, as well as the local fibrosis process. Integration of the vaginal and skin epithelia was made with no alterations in their primary characteristics.

Conclusion: The use of two skin flaps of the lower abdominal wall as a vaginal conduit presented good integration between skin and vaginal tissue with minimal length retraction, kept the patency during evaluations and did not show strictures, presenting good local healing and a low rate of complications.

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1. Introduction

Congenital vaginal agenesis is a deformity of embryonic origin that results from the absence of the mesonephric duct (Muellerian anomaly), with an incidence of around 1–5000 live-born subjects [1]. The main diagnosis involving this disease is the Mayer-Rokitansky-Küster-Hauser syndrome, where patients present chromosomally as 46 XX. The specific mechanism leading to vaginal development failure has not been elucidated: multiple events can promote or interfere in this process. The main differential diagnosis of this pathology is testicular feminization syndrome (androgen resistance), which has an incidence of 1 in 20,000 live-born subjects and affects patients with XY chromosomes [2].

Previous vaginal reconstruction procedures based on skin flaps and bowel segments use moulds to create and maintain space for the neo-vagina, which creates discomfort in these patients [3]. Vaginoplasty with intestine has a mucous secretion that increases the discomfort and has a higher morbidity [4,5]. The currently described techniques based on skin flaps have, as a drawback, the fact that they produce an extensive scar in the donor area and excessive hair in the flap region [6,7].

Vaginal reconstruction remains a formidable challenge in the reconstructive surgery area, not only due to the difficulty in obtaining satisfactory results in late follow-up, but also because of the important emotional impact of the diagnosis of a vaginal defect or absence in any woman. Our group has been studying the use of transverse skin flaps of the lower abdomen for different clinical indications, such as the creation of neophallus in aphaelia [8] and also creation of catheterized conduits used according to the Mitrofanoff principle [9].

In this context, we investigated in this experimental model the possibility that these flaps, having inferior and circumflex epigastric artery branches as their vascular bases, could be used as basis for a neo-vagina. It is our belief that this method would have as potential advantages the fact that it produces in the donor
area a cosmetically acceptable transverse scar and does not require the use of bowel segments, thus reducing the procedure morbidity.

2. Material and methods

We selected the rabbit as experimental model due to the familiarity of our group with this model in previous studies. The experimental protocol was reviewed and approved by the University Research Ethics Board. A total of 16 female New Zealand rabbits, aged 8 weeks old and with average weight from 2.5 to 3 kg was operated.

The animal was anesthetized with ketamine (30 mg/kg) and xylazine (5 mg/kg) intramuscularly. All animals were operated under sterile conditions. The technique consisted of delimiting two rectangular flaps (1 cm × 3 cm), opposed from each other in the midline of the lower abdominal wall (Fig. 1A). Vascular irrigation of the flaps was maintained by branches of the superficial epigastric and superficial circumflex iliac arteries (Fig. 2). The measurements were decided in a pilot experiment, after opening the vaginal cavity and using urinary plastic catheters to estimate vaginal diameter. This catheter was used in the skin to define the width of the flap (1 cm). The length was decided by measuring the rabbit vagina before excising the uterus and distal vagina. The lateral and cranial surfaces of the flaps were completely sectioned, providing them with enough mobility, which allowed a 90-degree rotation, leaving them in an upright position (Fig. 1B). The medial edges of the rotated flaps were anastomosed in a continuous suture with 5.0 polyglycolic acid suture thread forming a skin plaque (Fig. 1C). The next step was the anastomosis of the lateral edges of the flaps, using a plastic tube of 8 Fr from the mould and thus forming a cylindrical duct (Fig. 1D).

Then, we proceeded to abdominal cavity opening and excision of uterus and 1/3 distal of the vagina of the animal, in an attempt to mimic vaginal agenesis (Fig. 3A). We anastomosed the proximal end of the conduit to the remaining vaginal stump with separated points of polyglycolic acid 4.0 around 6–8 points: it was easy for this duct to reach the animal’s pelvic region (Fig. 3B and C). Finally, we closed the distal conduit stump (neo-vagina vault) with separate sutures of 4.0 polyglycolic acid and we fixed this stumps to the internal edge of the abdominal wall. Then, the abdominal wall was closed in a continuous suture with 3.0 polyglycolic acid and skin with 2.0 polyglycolic acid. No type of mould was left between the vagina and the conduit. The animals were kept in a heated room with ventilatory support until they were well awake. The experimental study consisted of 16 rabbits, divided into 4 groups according to the date of euthanasia at 2, 4, 8 and 12 weeks (each group = 4 rabbits).

At the time of euthanasia, the skin conduit and the remaining vaginal stump were removed in a block and catheterized with a plastic mould of 8 Fr to evaluate the conduit patency and circular Anastomosis. The piece was sectioned longitudinally to macroscopically evaluate the anastomosis region between the skin duct and vaginal stump (Fig. 4A–C). After that, the length of the skin conduit was measured with a numbered ruler (Fig. 4D). Subsequently, the piece was fixed in 10% formalin. The fixed parts were sectioned longitudinally, to evaluate histologically the integration between skin flap and vaginal mucosa, through hematoxylin and eosin and Masson trichrome staining. An experienced pathologist (RD) also evaluated the inflammatory process and the presence of subepithelial fibrosis, as well as possible changes in the vaginal mucosa and skin flap.

The inflammatory reaction was classified as acute when infiltrated by polymorphonuclear cells, and chronic when infiltrated by lymphomononuclear cells. A method of semi-quantitative measurement graduated from 0 to 4 (0, no inflammatory infiltrate; 1, minimal inflammatory infiltrate; 2, moderate inflammation; 3, major inflammation; and 4, with aggregated leucocytes/lymphocytes forming a microabscess was used. The level of sub-epithelial fibrosis was also analyzed semi-quantitatively, graduated from 0 to 3, with 0, no presence of fibrosis; 1, minimal presence of fibrosis; 2, moderate fibrosis; and 3, major presence of fibrosis. Quantitative analysis was performed evaluating the sub-epithelial fibrosis thickness in the anastomosis region through a microscopic graduated ruler in a randomized animal from each group (Fig. 5A). These criteria have been previously reported in another experimental study of the group [8].

Statistical evaluation of the final length of the skin flap was performed with the statistical software SPSS (Statistical Package for Social Sciences) version 11.0 for Windows, using the Wilcoxon test for paired samples, considering significant a p < 0.05.

Fig. 1. (A) Definition of two rectangular flaps (1 cm × 3 cm), that undergo a 90-degree rotation (B) to be sutured as a channel (C and D).
3. Results

3.1. Macroscopic evaluation

Only one case had partial dehiscence of the surgical wound, without complications and damage of the flaps. All pieces were shown to be open on analysis through an 8 Fr plastic probe. No narrowing point was noticed, denoting that all pieces were patent (Fig. 4).

The skin integration with vaginal tissue presented a quite solid appearance and without important retraction after removing the piece from the animal. There was no statistically significant retraction in any group (Table 1).

3.2. Microscopic evaluation

The skin conduit did not show significant changes, with local erosions, hyperkeratosis and acanthosis in most parts of the study.

Fig. 2. The pedicle of the flap is maintained by branches of the superficial epigastric and superficial circumflex iliac arteries.

Fig. 3. (A) Excision of uterus and distal 1/3 of the vagina and anastomosis of the proximal end of the conduit to the remaining vaginal stump (B and C).
follow-up, without interference in the flap structure. Only in the later group, an atrophy of the skin flap was observed, without compromising the epithelial integrity.

The vaginal mucosa did not present significant changes in the initial follow-ups. Only in the later follow-ups was keratinizing squamous metaplasia observed.

The inflammatory processes and fibrosis presented initially in an intense manner, but then we observed a progressive reduction mainly in the anastomotic region between skin flap and vaginal mucosa, showing a good integration between the pieces (Fig. 5C and D).

4. Discussion

Vaginal reconstruction is a great challenge in reconstructive surgery. The treatment of these patients should be individualized and consists of less aggressive methods such as non-surgical dilatation and many other surgical methods adopted in the creation of a neo-vagina. The main goal of a vaginoplasty is its creation without excessive morbidity, which shows a satisfactory appearance and that is functional. Even though multiple methods are available in the literature, a method that can be considered as “optimal” is still being looked for [10].

Among non-surgical methods and dilatation, we mention the method developed by Frank in 1938 that proved to be inadequate at long term, as did other procedures that follow the same principle, such as that by Ingram and Vecchietti [12–14]. The use of bowel segment for vaginal reconstruction, first reported by Baldwin in 1904, has as a negative factor a higher morbidity, even though more recent studies showed a development of the technique [15,16]. A limiting factor of this technique is excessive production of mucus in some patients [17,18]. However, the need to use a mould post-surgery, as well as in the procedure of McIndoe, which used a skin flap, leads to great discomfort in patients using this technique. Another complication of techniques based on skin flap is the presence of dyspareunia due to low lubrication and the risk of vaginal conduit stenosis [19].

The use of a myocutaneous flap, despite good technical viability, adversely affects the donor area, making this technique prohibitive in several cases, and reserved only for oncology cases [20]. The procedure by Chen et al. and Xiong et al., also based on flaps of the lower abdominal wall, presents a good functional result, but the esthetical aspect is unsatisfactory [21,22]. The procedure that used a pudendal-thigh flap, described by Wee and Joseph, has as drawback the presence of hair in the vaginal conduit [23,24].

In this study, we tried to evaluate an alternative method with the use of skin flaps of the lower abdominal wall in the creation of a neo-vagina in animal model. This study is based on a line of research with the application of skin flaps of the lower abdomen. Barbosa et al. evaluated the histology of this skin flap of the inferior abdominal region in the creation of a neo-penis in rabbits, showing satisfactory results [8]. Another study in this same research line was the creation of a catheterizable conduit using the same skin flap described by Rosito, Pires and Macedo and applied as an alternative to the appendix on the Mitrofanoff principle [9]. The authors found very satisfactory results in the animal model and applied it in three children, with a good result in the first year of follow-up.

The present study suggests a third possible application of these flaps of lower abdomen in creating a conduit to be used as neo-vagina. The technical viability was proved in this experimental study. No stenosis was identified in the conduit in any animal of the four evaluated groups, even without using a mould. Taking into account the absence of stenosis without the necessity for a mould

<table>
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<td>Skin flap measurements (length) on macroscopical evaluation.</td>
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<td>Group</td>
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<tr>
<td>Rabbit 1</td>
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<tr>
<td>Rabbit 2</td>
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<td>Rabbit 3</td>
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<td>Mean</td>
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p = 0.941.
and the site of removal of the flaps being of easy access and healing, the technique has become potentially attractive. The macroscopically evaluated conduit showed a slight contracture in its extension, even if not statistically significant, which probably would not interfere in the vaginal functional depth in the animal model. It is known that vaginal depth is important to satisfy the patient in sexual activity [11]. The flap also showed a good integration with the vaginal stump, observing an inflammatory process in the early phase that has become minimal in the later segments without any damage for the healing of the neo-vagina. Thus, we believe that the technique presented can become a good alternative of neo-vaginoplasty of analogous form to that found in the studied animal model.

We are aware of the limitations of our study, since good experimental findings cannot always be translated into satisfactory clinical results. Nevertheless, for a specific situation the technique can present advantages. The next step of the project will be to design a specific protocol for application in clinical situations that demand neo-vaginoplasty in humans.

5. Conclusion

The use of the two skin flaps of the lower abdominal wall as a vaginal conduit presented good skin integration with vaginal tissue with minimal retraction in the length of the neo-vagina, kept completely its patency and did not show stenosis, presenting good local healing and low rate of complications.

References