A modified single-armed microsurgical vasoepididymostomy for epididymal obstructive azoospermia: intraoperative choice and postoperative consideration

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Abstract

Background To evaluate the clinical outcomes and the time of sperm returning to the ejaculate after a modified single-armed 2-suture longitudinal intussusception vasoepididymostomy (SA-LIVE). Methods From March 2015 to December 2018, 134 patients with epididymal obstruction azoospermia underwent the modified single-armed vasoepididymostomy at Shanghai General Hospital. The outcomes and clinical findings were documented and evaluated. Results: The mean follow-up period was 17 months (range 3-36 months). Patency was assessed by the return of sperm in the ejaculate. The overall patency rate is 55.2 %, and the patency rates were 58.9%, 40.7%, 36.4%, and 58.9% for bilateral surgery, unilateral surgery, proximal anastomosis and distal anastomosis, respectively. The average time it took to achieve patency is 4.11±2.74 months. And in the first six months, 87.8% (65/74) patency patients were reported to found sperm in the ejaculate. The overall pregnancy rate was 40.9 % (29/66) at the follow-up of 3 to 36 months, and the natural pregnancy rate was 30.3 % (20/66). The natural pregnancy rate was 32.1% for bilateral surgery, 33.3% for the site of distal anastomosis but surprisingly, it was 0% for the site of proximal anastomosis. Conclusion: Modified SA-LIVE is safe and may achieve favorable patency and pregnancy rates. When double-armed sutures are not accessible, single-armed ones may be a good choice. The expecting patency time is about within one year. Assisting with intraoperative sperm cryopreservation, patients can achieve better pregnancy outcomes. Moreover, the low natural pregnancy rate for the proximal anastomosis suggested that we should reconsider the indications of SA-LIVE for EOA patients.

Key words: obstructive azoospermia; male infertility; vasoepididymostomy; patency; pregnancy
Background

Azoospermia affects 1% of the general population and 10-15% of infertile men[1]. Obstructive azoospermia, which could be raised for various reasons, is diagnosed in approximately 40% of azoospermic men[2], and epididymal obstruction is one of the most common reasons that caused obstructive azoospermia[3]. Obstructive azoospermia is one of the few correctable causes of male infertility by microsurgery. Microsurgical vasoepididymostomy (VE) has been established as a more cost-effective option for men with obstructive azoospermia than direct assisted reproductive techniques (ART)[4]. Furthermore, some chromosomal and genetic abnormalities can be filtered through natural pregnancy initiated with surgical correction, and epididymal damage that may happen during sperm retrieval could also be prevented[5]. Microsurgical VE demands superior surgical skills and meticulous surgical technique. Cornell first reported the single-armed 2-suture longitudinal intussusception (SA-LIVE), it was comparable to the double-armed procedure in an animal study[6]. Later, Zhao et al. reported their modified single-armed VE technique with favorable patency in a human study trial[7].

The success of microsurgical VE, except for surgeon’s microsurgical skill and extensive microsurgical training and clinical experience, also depends on the individual patient to some degree, which could be rather crucial in certain circumstances, thus makes it unpredictable. Previous studies have shown that a high patency rate may be related to certain factors, such as epididymal fullness, unilateral or bilateral procedure, site of anastomosis[8]. SA-LIVE may also achieve a high patency rate. Also, sperm collected during microsurgical VE should take cryopreservation for future usage in ART in case of surgical failure[9]. And in this cohort, we learned something new about choices of intraoperative procedures and decision-making post-operation.
Methods

Patient history

During the past 3 years, there are 158 patients who suffered from obstructive azoospermia underwent VE surgery in our center in total, however, during our follow-up, we only managed to collect the data of semen analysis of 134 patients from our center or from telephone follow-up (eight patients never went back to hospital to do the semen analysis post-operation, 16 patients were lost follow-up), and the pregnancy data of 66 patients among the 74 who achieved patency (2 patients aren’t married, and 6 patients were lost follow-up). In this cohort, 80/134 (55.9%) of the patients had previous history of urological or genital infection before the study, with 54 of them had medical records of being diagnosed with epididymitis. The etiology of the other 64 cases was unknown. No chromosomal or sex chromosomal abnormalities were detected in any patients. And no patients had vasectomy in this study.

Subjects and laboratory examination

From March 2015 to December 2018, we prospectively evaluated the patients diagnosed as epididymal obstruction azoospermia (EOA) without a history of vasectomy. All patients underwent semen analysis at least three times before surgery. No sperm was identified in a centrifugal (1,500×g) semen assay and ejaculate fructose tests were positive. The serum sex hormone levels, such as follicle-stimulating hormone (FSH) and testosterone (T), were within normal limits. Scrotal ultrasonography was tested to show dilation of the epididymal tube if present. These were objective measurements for obstructive azoospermia[3].

Diagnostic clinical condition and criteria

The diagnostic criteria for inclusion was briefly described as follows[10]: obstruction was suspected when the infertile male had normal ejaculate volume with azoospermia; the
physical examination showed non-atrophic testes with normal vas deferens bilaterally, slightly swollen epididymis and bilateral or unilateral hard epididymal nodules; normal serum total T and FSH levels; the ultrasonography showed the dilation of epididymal tube, without dilation of the ejaculatory duct or seminal vesicle. The patients with chromosomal or sex chromosomal abnormalities, history of vasectomy, or whose female partners were reported to be infertile, were excluded from our study.

**Surgical procedure**

1. General Preparation: All patients underwent scrotal exploration under general anesthesia. Surgeries were performed by two same experienced surgeons. Testis was exposed, and normal spermatogenesis was confirmed by testicular biopsy during the operation. All patients provided written informed consent to be in the study with guarantees of confidentiality.

2. Microsurgical preparation: Use of a 24-gauge angio-catheter sheath to cannulate the lumen of the vas and Typan blue was injected into the vas deferens to judge the patency of the seminal vesicle side. A sufficient length should be freed to allow the most proximal convoluted portion of the vas to be later brought to the lateral aspect against the epididymis without tension.

3. Microsurgical SA-LIVE approach: A Carl Zeiss S88 operating microscope (Carl Zeiss Shanghai Co., Ltd., Shanghai, China) was used to perform the microsurgical procedure at a magnification of 8–15. A dilative epididymal tubule was chosen under microscope guidance, and carefully dissect the single dilated tubule. Then anastomosis was performed using the modified SA-LIVE technique. Two single-armed 10–0 nylon sutures (Ethicon W2790, length 200 mm, circle 3/8) were prepared for the intussusception sutures. The first suture was placed in an outside-in fashion through the mucosal layer of the vas deferens at point a1. Then, the needle was used to pierce the lateral aspect of the
epididymal tubule and was placed longitudinally. The second 10-0 single-armed proline suture was placed identically through point b1 on the vas deferens, parallel to the first suture on the contralateral side of the epididymal tubule. The two needles were used to parallelly pierce the lateral aspect of the epididymal tubule both within the tubule itself and out longitudinally. The epididymal tubule was opened longitudinally between the two sutures with a micro knife, and the exuded epididymal fluid was examined for sperm. If sperm or sperm fragments were present, the needles were pulled through the wall of the epididymis and placed an inside-out manner through the full layer of the vas at position a2 and b2 (Figure 1). Finally, all the sutures were tied together (a1 to a2 and b1 to b2), the epididymal tubule was gently intussuscepted into the lumen of the vas deferens. The epididymal tunic was then secured to the vassal muscle and adventitia with an 8-0 nylon suture. Epididymal and testicular sperm was cryopreserved during VE as a backup of VE.

Postoperative management
Most of the patients were discharged home the first day after the surgery, and they were advised to refrain from any heavy lifting and sports activity for eight weeks after surgery. Sexual abstinence instructed for four weeks. Semen analyses were initiated at four weeks after surgery, and every month after that until the pregnancy was achieved. Patency was defined as the presence of motile sperm in the ejaculate on at least one postoperative ejaculate sample. Pregnancy was defined as the establishment of a viable pregnancy. The follow-up information was obtained by clinic visits and telephone contacts. Patients without a postoperative semen analysis or patients who were lost to follow-up were excluded from the report, and the follow-up time is at least three months.

Statistical analysis
The patency rate and pregnancy rate of each group (Unilateral and bilateral Microsurgical VE group proximal and distal anastomosis group) were calculated. The Chi-square test
was used for all analyses. Significance was defined by P < 0.05.

Results

From March 2015 to December 2018, 158 patients diagnosed with an epididymal obstruction underwent modified SA-LIVE in our center, and among which 134 patients were followed prospectively. The mean age was 32.1±6.7 years (ranged 23–50), and the mean follow-up was 17±3.3 months (ranged 3–36). The mean FSH and T level were 4.3±2.4 mIU/mL and 5.0±2.9 ng/mL. The mean testicular size measured by ultrasonography was 15.4±3.3 cm³ (Table 1).

Sperm was present in the ejaculate of 74 of 134 (55.2%) patients after the surgery, and the average time it took to achieve patency was 4.11±2.74 months. And in the first six months, 87.8% (65/74) patency patients were reported to found sperm in their ejaculate. Only 12.2% (9/74) patients claimed to achieve patency in the next six months (Figure 2). The variables related to patency for follow-up are listed in Table 2. The patency rates were 58.9%, 40.7%, 36.4%, and 58.9% for bilateral surgery, unilateral surgery, proximal anastomosis and distal anastomosis, respectively. In this cohort, 30.3% (20/66) patients were reported to have spontaneous pregnancy after VE. The variables related to natural pregnancy are listed in Table 3. The natural pregnancy rates were 30.5% for bilateral surgery, 20% for unilateral surgery, 33.3% for the site of distal anastomosis, respectively. But surprisingly, it was 0% for the proximal anastomosis. When the variable was measured in years, from 2015 to 2018, the patency rates were 42.1%, 58.3%, 53.3% and 61.1% for each year; the natural pregnancy rates were 37.5%, 32%, 50% and 15%, respectively. Furthermore, nine couples got pregnant by intracytoplasmic sperm injection (ICSI) (seven used frozen sperm collected during operation, and two used fresh sperm from ejaculate). The total and natural pregnancy rates were 43.9% (29/66) and 30.3% (20/66) at the
follow-up of 3 to 36 months, respectively.

Discussion

Approximately 10–15 % of infertile men suffer from azoospermia, while about 40% of them suffer from obstructive azoospermia (OA). The obstruction may be due to bilateral occlusion at any point of the reproductive ductal system which comprises the efferent duct, epididymis, vas deferens, and the ejaculatory ducts. And microsurgical anastomosis is considered as the most successful measure to get a reversal, including microsurgical vasovasostomy (VV), cross vasovasostomy (cVV), and vasoepididymostomy (VE) [11]. Here we are about to discuss the modified single-armed 2-suture longitudinal intussusception vasoepididymostomy. Our data are comparable to the previously reported patency rate of 52%–92% and the pregnancy rate of 11%–56%[12, 13]. In the cohort, we evaluated pre-, intra- and postoperative variables of individual patients that might affect the outcomes of the modified SA-LIVE.

Unlike the USA, EOA is rarely caused by vasectomy but by infection in China[14, 15]. Among our 134 patients with obstructive azoospermia, there are 80 patients reported to have a history of epididymitis or orchitis, full epididymis could be palpated easily and sometimes with inflated nodes. Their diagnostic ultrasonography findings also proved the existence of inflammation in the scrotum, which showed marked thin netlike ectasia of the epididymal tube with the inner diameter up to 0.4 mm.

As we know, VE with the double-armed suture is the standard golden management for the EOA, and LIVE simplifies the anastomosis and offers better outcomes[8, 16]. However, in some parts of world like China, these specialized double-armed sutures for male infertility microsurgery are challenging to obtain, and the access of single-armed microsurgical sutures seems much easier[17]. Furthermore, the cost of single-armed sutures is cheaper than the double-armed microsurgical sutures. Therefore, SA-LIVE should be concerned as
an effective alternative when double-armed sutures are not available. When the needles are in the tubule, the two sutures in a lower position can avoid the crossing of the sutures[7]. The two knots of the suture are left outside during the procedure, which may decrease the possibility of fibrosis and anastomotic stricture. However, using the single-armed suture does have a disadvantage of time-consuming compared with the double-armed one. The double-armed suture was placed inside-out on the vas deferens to avoid back-walling the tubular lumen. As we use the single-armed suture, the needle passed through the inferior points of the vasal mucosal layer in an outside-in fashion through the epididymal tubule, and finally through the superior points of the vasal mucosal layer in an inside-out fashion, which would consume double time than the double-armed one and increase the risks of surgery. Also, we have to dilate the vasal lumen to make it wide enough to pass the needle through the lumen with the aid of a microneedle holder to avoid back-walling during the suture placement, and these procedures must be completed before we can confirm the existence of sperm in the epididymal fluid[18]. If no sperm was found in the epididymal fluid, we might have to repeat this procedure 2 to 4 times, which would significantly increase the time required for surgery and the fatigue of the surgeon. In this cohort, the patency and the pregnancy rate were 55.2% and 40.9%, respectively. The patency rate of bilateral and unilateral surgery is 75.3% and 41.7%; the spontaneous pregnancy rate is 32.1% for patients who underwent bilateral surgery compared with 10.0% for unilateral cases. When it came to the site of the anastomosis, the patency rate is 36.4% and 58.9% for proximal and distal of anastomosis of the epididymis, respectively. Our data are comparable to the previously reported patency and pregnancy rates. However, no statistically significant associations were found between the patency rate and various predictors, such as bilateral or unilateral anastomosis, anastomotic site, etc. (P > 0.05; Table 2 & Table 3). What’s more, there is an astonishing outcome that the natural
pregnancy rate is 0% for the proximal anastomosis group, while it’s 33.3% for the distal group, which makes us take further consideration of whether patients can benefit from the proximal anastomosis. Firstly, the luminal diameters of epididymal tubules in the caput are significantly smaller than those in the corpus and caudal, so the modified SA-LIVE on the distal epididymis is more accessible than that on the proximal epididymis. Moreover, spermatozoa acquire the capacity to become fully motile as well as the ability to recognize and fertilize an egg within the epididymis, which may improve the pregnancy rate. As the sample size is not large enough to perform a further investigation, the reason for their failure is still to be determined.

Although there have been many kinds of clinical research on VE, no researchers gave out a specific follow-up time frame on SA-LIVE. Our study revealed that there were 87.8% (65/74) patients claimed to find sperm in their ejaculate within six months post-operation, and only 12.2% (9/74) claimed to achieve patency in the next six months. And no patient achieved patency since then, which indicate that it may take no more than 12 months for the patients to achieve patency and the follow-up time of SA-LIVE on patency can set the limitation up to 12 months. The average time was 4.11±2.74 months, the success rate for the first six months is 48.5% (65/134), and 11.4% (9/69) for the next six months. This could be of great value for the clinical doctors and researchers to predict the outcome of patients taking SA-LIVE as well as the proper time to transfer to ART.

It’s worth mentioning that there were four patients diagnosed as non-obstructive azoospermia in other hospitals previously according to the negative results of the testis histopathology, however, we surprisingly found motile sperm through the routine testicular biopsy. Thus, the scheduled operation of micro-dissection transferred to VE, and three of the four achieved patency afterwards. This suggested that the testis biopsy during the operation is quite necessary[19].
Also, we had one patient underwent VE in another hospital previously, and we performed the repeated VE operation due to the first failure. And this time, sperm appeared in the ejaculate, and finally this couple achieved a spontaneous pregnancy. We may conclude that other than ICSI, a second VE surgery may be a good choice for those patients who failed the first one.

VE is also an effective treatment for azoospermic patients with epididymal obstruction and prior failure to achieve pregnancy by sperm retrieval with ICSI[20]. Three patients diagnosed with OA chose to use the sperm from testicular puncture for ICSI in another hospital, unfortunately failed, maybe due to the miscarriage, maturation arrest in utero or the failure of embryo transfer[20]. After taking SA-LIVE in our hospital, all of them achieved patency, and one patient had a natural pregnancy, one patient underwent IVF after the surgery, and the last one is still trying to get a natural pregnancy. As known to us all, microsurgical vasoepididymostomy do have significant advantages, like costs saving, spontaneous pregnancy possibilities, and decreasing potential risks of congenital disability comparing with ICSI. Thus, microsurgical reconstruction is an effective treatment and should be the first choice for epididymal obstruction patients whose female partners have normal fertility features. EOA patients with prior failure of ICSI could take consideration of taking LIVE to acquire pregnancy.

Our research revealed that if sperm didn’t show up in the ejaculate after one-year post-operation, ART might be the remedial choice for these patients. Thus, intra- or post-operative sperm cryopreservation will be helpful. Intraoperative sperm cryopreservation could avoid the additional surgeries for sperm retrieval in case of the failure of microsurgery. Post-operative sperm cryopreservation allows sperm from the ejaculate to be used for ART when late failure happens[14]. In our cohort, we found out that one patient who achieved recanalization of the vas a got the reoccurrence of obstruction six
months later. The recurrence rate of obstruction is 0.07% in our center, which is in line with the rate reported previously[21]. In the present series, sperm cryopreservation was used for a total of 132 patients. 29 patients who didn’t get vas patency achieved pregnancy through ICSI by the sperm cryopreserved intraoperation, and nine successful patients choose to use the sperm from ejaculate to have babies through ART. The pregnancy rate was improved from 30.3% to 43.9%. As the ART technique has been developed in few decades, the influence of whether the sperm came from testis or post-epididymis on the next generation stayed unclear, yet these figures are waiting to be told by the to-be-grown-up babies. After all, we advocate intra- or postoperative sperm cryopreservation for all OA patients.

Conclusion: Modified SA-LIVE is instead a good choice when double-armed sutures are not accessible. The expecting patency time is within one-year, ART may be the remedial choice for patients. Assisting with intra-operative sperm cryopreservation, patients can achieve better pregnancy outcomes. The low natural pregnancy rate for the proximal anastomosis suggested that we should reconsider the indications of SA-LIVE for OA patients.

Abbreviations

SA-LIVE: modified single-armed 2-suture longitudinal intussusception vasoepididymostomy
VE:Microsurgical vasoepididymostomy
ART: assisted reproductive techniques
FSH: follicle-stimulating hormone
T: testosterone
VV: vasovasostomy
ICSI: intracytoplasmic sperm injection
OA: obstructive azoospermia
EOA: epididymal obstructive azoospermia

IVF: in-vitro fertilization

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving human participants were in accordance with the ethical standards of Institutional Ethical Review Committee of Shanghai general hospital, Shanghai Jiaotong University School of Medicine and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors’ contributions: Peng Li, Erlei Zhi, Chencheng Yao, Chao Yang, Liangyu Zhao, Ruhui Tian, Huixing Chen, and Yuhua Huang performed the surgery, Yuexin Yu and Nachuan Liu collected the data. Nachuan Liu and Peng Li analyzed the data and wrote the manuscript. Zheng Li conceived the project, designed the analysis, and supervised the entire study.

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**Tables**

**Table 1:** Preoperative characteristics and intraoperative choices in all 134 patients

| Items                             | Value                          |
|-----------------------------------|-------------------------------|
| Age (year), mean                  |                               |
| Patients                          | 32.1±6.7 (ranged 23-50)       |
| Female partners                   | 27.2±3.7 (ranged 20-43)       |
| Serum FSH (mIU/mL), mean          | 4.3±2.4                       |
| Serum total testosterone (ng/mL), mean | 5.0±2.9                    |
| Testicular size (cm³), mean       | 15.4±3.3                      |
| Surgery, n (%)                    |                               |
| Bilateral                         | 107(79.9%)                    |
| Unilateral                        | 27(20.1%)                     |
| Anastomotic site, n (%)           |                               |
| Proximal                          | 22(16.4%)                     |
| Distal                            | 112(83.6%)                    |

**Table 2.** The patency rate stratified by two variables for 134 patients.

| Variable                      | Patency (n)     | P Value |
|-------------------------------|-----------------|---------|
| Surgery                       |                 | 0.090   |
| Bilateral                     | 63/107(58.9%)   |         |
| Unilateral                    | 11/27(40.7%)    |         |
| Anastomosis site              |                 | 0.052   |
| Proximal                      | 8/22(36.4%)     |         |
| Distal                        | 66/112(58.9%)   |         |

**Table 3.** The natural pregnancy rate stratified by two variables for 134 patients.
| Variable          | Natural pregnancy (n) | P Value |
|-------------------|-----------------------|---------|
| Surgery           |                       | 0.442   |
| Bilateral         | 18/56 (32.1%)         |         |
| Unilateral        | 2/10 (20.0%)          |         |
| Anastomosis site  |                       | 0.090   |
| Proximal          | 0/6 (0%)              |         |
| Distal            | 20/60 (33.3%)         |         |

**Figures**
Figure 1

Placement of sutures in modified SA-LIVE. The needles were sequentially placed outside-in (a1 and b1) through the mucosal layer of the vas deferens, parallelly through the epididymal tubule, then placed inside-out (a2 and b2) through the mucosal layer of the vas deferens.
Figure 2

Time of sperm returning to the ejaculate. The column height of the histogram represents the patients counts within various periods to achieve patency.