Review Article

Male infertility microsurgical training

Akanksha Mehta[1], Philip S. Li[2], Marc Goldstein[2]

[1]Department of Urology, Emory University School of Medicine, 1365 Clifton Road NE, Building B, Suite 1400, Atlanta, GA 30322, USA; [2]Department of Urology, Weill Cornell Medical College, 525 East 68th Street, New York, NY 10065, USA

Correspondence to: Akanksha Mehta, MD. 1365 Clifton Road NE, Building B, Suite 1400, Atlanta, GA 30309, USA. Email: akanksha.mehta@emory.edu.

Abstract: A strong foundation in microsurgical techniques is imperative for urologists and clinical andrologists specializing in male infertility. Laboratory-based microsurgical training enhances surgical skills, improves surgeon confidence, and reduces stress and operating time, thereby benefiting both the patient and the surgeon. The laboratory environment additionally allows for the development of novel and innovative techniques. This review provides guidelines for setting up a microsurgical laboratory to develop and enhance microsurgical skills using synthetic and animal models.

Keywords: Microsurgery; surgical training; surgery laboratory; surgical techniques

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Introduction

A strong foundation in microsurgical techniques is imperative for urologists and clinical andrologists specializing in male infertility. However, developing good microsurgical skills can be challenging, even for surgeons who are skilled in conventional open and laparoscopic surgical techniques. The use of an operating microscope dramatically changes the scale of surgery, and alters the surgeon's spatial perception. The use of fine microsurgical instruments and suture materials introduces novel ergonomic considerations. Coordination, manual dexterity, and steadiness of movement under the microscope require practice and time to achieve.

Success in male infertility microsurgery is heavily dependent on surgical skill. Unlike conventional open and laparoscopic surgery, where clinical outcomes are readily apparent either intra-operatively or in the immediate post-operative period, patient outcomes following microsurgery are often not apparent for several months after the procedure. As an example, return of sperm to the ejaculate following vasovasostomy or vasoepididymostomy may take up to 12 months after surgery in some cases. Male infertility microsurgery, therefore, ranks amongst the most technically and mentally challenging of surgical procedures.

Surgical education has traditionally been built on the Halstedian apprenticeship model, where experts teach skills to surgeons-in-training using real patients (1). However, in the modern era of healthcare, increasing pressures on operating room time and resources have forced us to re-examine the role of the operating room as the exclusive venue for teaching surgical skills (2). Efforts to improve the duty-hours of surgeons-in-training, have, unfortunately, further limited operative training time. As a result, there is increasing interest in the development of surgical skills training laboratories, across various surgical disciplines. In a prospective study of surgical residents, hands-on laboratory-based surgical training translated into better surgical performance compared to didactic training alone (3). Laboratory training was also associated with better retention of technical skills by novice surgeons over a 4-month follow-up period (3).

Cognitive and technical competency in microsurgery are essential before entering the operating room, and are best established through repeated practice in a microsurgical training laboratory. The aim of this article is to provide guidelines for setting up a microsurgical laboratory and to review basic microsurgical skills using synthetic and animal models.

The microsurgery laboratory

Establishing a microsurgical laboratory does not necessitate the purchase of elaborate or expensive equipment, besides

an adequate operating microscope and basic microsurgical instruments. Synthetic practice materials, such as suturing practice cards and silicone tubing, are commercially available, inexpensive models for developing basic microsurgical skills (4). Advanced skills for vasovasostomy and vasoepididymostomy can be acquired using human vas segments, or small animals such as rats. However, live animal surgery requires access to an established, animal care facility, which can be expensive.

Operating microscope

There are a variety of operating microscopes available for use in the laboratory. The most important characteristics to consider when selecting a microscope are bright illumination, a smooth focusing system, and ease of operation. For male infertility microsurgery, a magnification range between 10× and 25× is sufficient. Either a motorized or a manual zoom and focus system may be used for training purposes. The basic components of an operating microscope consist of the objective lens, eyepieces, binocular tube, and microscope body, which houses the magnification changer. The objective lens determines the focal length or working distance. Male infertility microsurgical procedures are usually performed using a 200 mm objective length, which focuses on the surgical field 200 mm away from the objective lens. Optimal set-up of the objective length minimizes physical strain to the surgeon, and allows for easy handing of instruments under the microscope.

The operating microscope should provide a stable and stationary field of view. In the laboratory, this can be achieved by securing the microscope to a solid and stable working table, with workspace dimensions of at least 30 in. ×24 in. Ensuring a table height of at least 30 in. allows for comfortable placement of the operators knees under the table. An adjustable stool allows the operator to modify his or her height as necessary. If applicable, foot pedals controlling the zoom and focus should be placed in a location that the operator can easily access, while maintaining his or her focus through the eyepieces.

Operating microscopes with two binocular heads are optimal for surgical training purposes, as these allow for an instructor to observe and provide guidance and feedback to the trainee.

Microsurgical instruments

Although a variety of specialized microsurgical instruments are commercially available, most urological microsurgical procedures can be performed using the select microsurgical instruments listed in *Table 1*. *Table 2* lists additional surgical supplies that are useful for establishing a microsurgical laboratory equipped for performing small animal surgery.

Microsurgical instruments are delicate in design and susceptible to damage, not only during use, but also during cleaning. Microsurgical instruments should be handled with care, and stored in a specially designed instrument case. Each instrument should be examined under the microscope prior to use. If necessary, instrument tips can be sharpened and repaired using Arkansas oil stone or emery paper.

Following use, instruments should be soaked in a hemolytic enzyme solution and then rinsed with water for careful cleaning. Damage to instruments is most likely to occur during cleaning. Once dry, the fine instrument tips should be protected by plastic covers or segments of silicone tubing prior to storage. Contact with other metal objects can cause the instruments to become magnetized. An inexpensive surgical instrument demagnetizer can be used to address this problem.

Basic preparation for microsurgery

In order to perform well, the microsurgeon should be familiar with his or her operating microscope and surgical instruments. A comfortable working environment should be created to ensure an ergonomic sitting position and to optimize manual dexterity. Mastering accurate microsurgical techniques requires patience and practice. Simple strategies such as adequate rest prior to a practice session, avoidance of heavy lifting, and minimization of mental stress can maximize the benefit from each practice session.

In the laboratory, surgeon comfort at the working table can be improved with the use of towels or foam to support the arms and hands. Maximum illumination with sharp focus should be utilized at all times under the microscope. Correcting the focus at the highest magnification ensures focus at all lower magnifications. The interocular distance of the eyepieces should be adjusted until a single image is visible through the binocular scope. The appropriate focal distance should be used.

The level of magnification should be adjusted as necessary during practice, as it is during surgical procedures. Low magnification should be used for tissue dissection and manipulation of the needle and suture materials. In contrast, high magnification is needed for preparation of the vasal ends or the epididymal tubules for re-anastomosis,

Table 1 Basic microsurgical instrument set

- Non-locking needle-holder with a rounded, fine-curved tip (13.5 of 15 cm in length)
- Straight, fine-tip forceps with suture platform (13.5 or 15 cm in length)
- Straight, fine tissue forceps with teeth (13.5 or 15 cm in length)
- Curved, blunt-tip dissecting scissors
- Sharp, Iris scissors
- Angled vessel dilator with a slender, tapered tip
- Vas approximator clamp for vasovasostomy and vasoepididymostomy (Ex: Goldstein microspike approximator; ASSI Inc., Westbury, NY, USA)
- Microsurgical bipolar cautery with fine-tipped forceps
- Non-sterile Sharpoint® microsurgical single-armed 9-0 and single- or double-armed 10-0 nylon sutures (Angiotech Pharmaceutical Inc., Vancouver, BC, Canada)
- Sharpoint® microsurgical suturing practice cards (Angiotech Pharmaceutical Inc., Vancouver, BC, Canada)
- Microtip surgical marking pen (Ex: Kendall Devon fine-tip surgical skin marker; Devon Industries, Buffalo, NY, USA)

Table 2 Non-microsurgical supplies

- Needle holder
- Smooth and toothed tissue forceps
- Suture scissors
- Curved dissecting scissors
- Surgical clip applier
- Operating board, at least 35×35 cm^2 in size
- Soft silicone tubing or preserved segments of vas deferens
- Tape for fixing practice objects to operating board
- 10 cc syringe and attached 27-guage angiocatheter for irrigation, with normal saline or lactated Ringer's solution
- Non-reflective drape to cover operative field and provide a satisfactory background, such as blue-colored paper drapes or towels
- Spasmolytics, such as 1% or 2% lidocaine hydrochloride (20 mg/mL)
- Heparin sulfate solution (100-150 units/mL), for use during vasovasal re-anastomosis in animal surgery
- Hemolytic enzyme cleaning solution, such as Haemo-Sol (Haemo-Sol Inc., Baltimore, MD, USA)
- Storage trays for microsurgical instruments
- Surgical instrument demagnetizer

and for passage of the needle through the lumen of the vas or epididymis. Following suture placement, surgical knots are best tied under low magnification.

Suturing technique

Proper hand positioning and instrument handling under the microscope is key to perfecting suturing techniques under the microscope. Most microsurgical tasks, including suturing and knot tying, require only a slight movement of the fingers. The goal should be to keep the hand still, with the thumb, index and middle finger supporting one another. As previously described, the use of towels to support the surgeon's hand and forearm minimizes fatigue, and can also serve to minimize hand tremors.

While many surgeons develop their own preferred way of holding microsurgical instruments, the pencil holding position, with the instrument resting between the thumb and index finger, is recommended. This position allows for controlled movement of the instrument while providing maximum ergonomic support for the surgeon's hand.

Urologic microsurgery utilizes fine 9-0 and 10-0 needles and suture material, which are easily damaged if held too firmly. Thus, developing a secure but gentle touch is

important when handling microsurgical instruments and suture materials. For maximal stability, the needle should be grasped by the needle holder at a point that is approximately one-half to two-thirds of the way away from the tip of the needle. Fine adjustments to the angle of the needle can be made by touching the needle with the tips of the forceps. The needle should always be manipulated under the optical magnification of the operating microscope.

The direction of the needle depends on whether a forehand or backhand stroke is planned. For most individuals, mastering the forehand stroke is considerably easier. However, being able to effectively handle and rotate the needle in any direction is essential for a microsurgeon, and developing this basic skill alone entails considerable patience and practice.

Micro-suturing practice card

Basic microsurgical suturing skills can be acquired using a practice suturing card (Angiotech Pharmaceutical Inc., Vancouver, BC, Canada), which is a simple and cost-effective tool. An "incision" is made on the latex surface of the card using a scalpel or microknife. The surgeon then places a series of interrupted stiches in order to close the incision in an efficient, precise, and atraumatic fashion. It is important to estimate the entrance and exit points prior to passing the needle through the card. These sites should be equidistant from the edge of the incision. The needle should pierce the latex "tissue" perpendicularly, at a point that is approximately two-thirds the thickness of the tissue. Microforceps should be used to apply gentle counter-pressure to help pass the needle through the tissue, one edge at a time. Movement of the surgeon's fingers must always follow the curve of the needle, in order to minimize trauma to both the tissue and the needle.

Non-sterile single-armed or double-armed 10-0 nylon sutures can be used for practice, as well as for vaso-vasal and vaso-epididymal anastomoses in animal models. These sutures are commercially available and are considerably less expensive than the 10-0 double-armed sutures that are used for surgical anastomoses in the operating room. The use of single-armed 10-0 nylon sutures to perform vasovasostomy in surgical settings where access to double armed suture is either not available, or is prohibitively expensive, has also been described in the literature (5).

Microsurgical knots

Male infertility microsurgical procedures often involve challenging anastomoses between discrepant luminal diameters, such as thick muscular walls of the vas deferens, and delicate, thin walls of the epididymal tubules. As a result, suture placement and knot tying can easily consume 40% to 65% of the total operative time (6), and operative success is greatly facilitated by the surgeon's ability to efficiently and securely tie microsurgical knots under the operating microscope.

Given the complexity of the anastomoses in male infertility microsurgery, and the level of precision required, most microsurgeons prefer a double-throw surgeon's knot as the first knot, followed by two or more single-throw square knots, to prevent suture unraveling. The first knot should be tightened until the tissue edges are just approximated, but not strangulated. Repeated practice with knot-tying in the microsurgical laboratory is important to learn the best technique for looping the suture around the surgical instruments, the necessary length needed for the short end of the suture, and appropriate amount of tension required to secure the knot without breaking the suture. Once the knot has been completed, the suture should be placed on gentle tension and cut under the microscope using sharp microscissors, leaving a length of 1-2 mm in order to prevent unraveling of the knots.

Online microsurgical videos are a useful educational resource for understanding and learning both basic suturing techniques, as well as more complex procedures in male infertility microsurgery. For more detail, the reader is referred to: www.maleinfertility.org.

Models for microsurgical training

Microsurgical vasovasostomy and vasoepididymostomy are amongst the most difficult anastomoses in microsurgery. Several different models for vasovasostomy and vasoepididymostomy training can be used in the laboratory, ranging from silicone tubing, to segments of human vas deferens, and live animals. A good model should allow not only for practicing suture placement techniques, but also for practicing and optimizing the set-up of the surgical field.

Silicone tubing

Soft silicone tubing is an inexpensive and effective alternative to the use of human specimens and live animals for microsurgical training. A 5-10 cm length of medical grade silicone tubing can be used to practice both the one-layer and two-layer techniques for an end-to-end

vasovasostomy. The tubing is held and stabilized on the surgical field with a Microspike approximator, which, in turn, is fixed with tape to the surgical field (7). The tubing is divided with a scalpel between the arms of the approximator in order to reveal its lumen in cross-section. Depending on the size of the tubing, the number of interrupted stiches required to complete the anastomosis should be estimated prior to embarking on suture placement. Marking the location of these stiches using a microtip surgical marking pen allows the surgeon to separate the act of planning stitch placement from their execution. This important step leads to greater concentration ability and accuracy (8).

The one-layer anastomosis technique is simpler than the multi-layer technique, and has the advantage of allowing the trainee to focus on proper position, order, and suture placement for the anastomosis.

The needle is passed perpendicular to the surface of the tubing, in the forehand direction, from the outside of the tubing towards the lumen. The entry site is a point approximately twice the wall thickness from the cut edge of the tubing. Counter-pressure applied using microforceps is used to guide the needle through the wall of the tubing, exiting in the lumen between the tips of the forceps. The needle is carefully pulled through the wall of the tubing following its curvature, using successive, gentle motions. The needle is then perpendicularly passed through the opposite wall of the tubing, from the lumen to the outside, exiting at a point that is equidistant from the cut edge of the tubing. The suture is securely tied, as described above, in order to approximate the edges of the tubing. Another two to four evenly-spaced stitches are placed on the remaining anterior wall of the tubing. The approximator is then turned over, and folded in the opposite direction, to expose the posterior wall of the tubing. The identical procedure is repeated on the second side. Normally, a single layer of eight to twelve interrupted nylon stiches are required to complete the anastomosis of the silicone tubing.

Preparation of the silicone tubing for the two-layer anastomosis is the same as that described for the one-layer technique. The inner layer of the tubing simulates the vasal mucosal layer. Two or three 10-0 nylon sutures are placed through the inner one half of the tubing wall to achieve mucosal approximation. If using single-armed suture, the stiches are both placed in the forehand direction. In contrast, if double-armed suture is used, forehand and backhand stiches are required in order for the suture knots to be tied outside the lumen of the tubing. Once the mucosal stiches have been tied, single-armed 9-0 nylon suture is used to place interrupted stiches through the wall of the silicone tubing, without penetrating the lumen of the tubing, exactly in-between the previously placed mucosal stiches. This step simulates approximation of the vasal muscularis and adventitial layers. Once the anterior half of the anastomosis is complete, the approximator is turned over to expose the posterior wall of the tubing. The identical procedure is repeated on the second side. The two-layer anastomosis usually requires six to eight interrupted 10-0 nylon stiches in the inner layer, and eight to twelve interrupted 9-0 nylon stiches placed in the outer layer of the tubing.

Vasectomy segments

The use of vasectomy segments for microsurgical training was originally reported by Belker *et al.* in 1978 (9). Vasal segments can be harvested at the time of vasectomy, or from radical prostatectomy specimens, with patient permission. Although the use of fresh tissues for practice is ideal, this can often be difficult to coordinate. Various techniques for the preservation of vasal segments have, therefore, been described (10). Specimens can be simply preserved in saline solution for a short period, but become "macerated" over time. Alternatively, they may be frozen at –20 ℃ after harvesting. However, the post-thaw quality of segments preserved in this fashion is variable. Freezing the specimens in saline or glycerol is associated with better mucosal and muscularis quality than freezing without media (10). Belker *et al.* additionally noted that vasal segments preserved in saline-soaked gauze, in an airtight container, in the refrigerator, could be preserved for up to eight weeks (9). Regardless of the preservation technique, it is important to remember to keep the vasal segments moistened with normal saline or lactated Ringer's irrigation when working under the microscope.

Although vasectomy segments can be used for one-layer or two-layer vasovasostomy training, the author recommends mastering the single-layer technique using silicone tubing, and reserving vas specimens for practicing the more difficult multi-layer anastomoses. Preparation of the vasal segments for re-anastomoses is similar to that described for the silicone tubing. A blue drape placed on the operating field acts as a contrast background. If the available vas segment is long in length, it should be secured in the Microspike approximator and sharply divided using a surgical knife between the arms of the approximator. If two shorter segments are used, the ends of these segments may need

to be refreshed with the surgical knife prior to placement in the approximator. The lumen of the vas deferens should be carefully inspected under 8-10× magnification. If the lumen is not clearly visible, a fine micro-vessel dilator may be gently inserted into the lumen and removed. Care should be taken not to damage the mucosal layer. Following successful dilation, a mucosal ring should be seen. To enhance visualization of the mucosal ring, indigo carmine dye may also be applied to the cut surface of the vas using a Q-tip or Weck-Cel sponge (Beaver-Visitec Inc., Waltham, MA, USA). Indigo carmine is preferable to methylene blue as it is non-toxic to sperm (11). The microtip marking pen is then used to place six evenly-spaced marks around the circumference of the vas along both vasal ends. The two vasal ends are aligned with respect to these markings.

Double-armed 10-nylon suture is used to place three interrupted stitches along the anterior wall of the vas deferens, in through the mucosa, and out through the muscularis layer. Once these are securely tied, good approximation of the mucosal layer should be appreciated. Two to four interrupted 9-0 adventitial sutures are placed in between the mucosal stitches to complete the second layer. The approximator is then turned over and the identical procedure is repeated on the posterior wall of the vas deferens. Six mucosal and six to ten adventitial stitches are usually sufficient for achieving a watertight anastomosis.

Five key principles should be followed to perform a successful vasal re-anastomosis: (I) an operating microscope should be used for optical magnification; (II) fine monofilament suture should be used to place interrupted stitches for the anastomosis; (III) blood supply to the vas deferens should be preserved; (IV) accurate mucosal approximation of the cut edges of the lumen of the vas deferens should be performed; and (V) a tension-free anastomosis should be created. The long-term patency of a vaso-vasal anastomosis can, of course, only be evaluated in a live animal model. However, a technically accurate anastomosis using silicone tubing or vasal segments should be leak-proof when injected with fluid, and have a patent lumen when observed in cross section under the microscope. Uniformity and accuracy of suture placement can also be assessed by longitudinally cutting open the mucosal and adventitial layers of the anastomosis to expose the lumen.

Live animal surgery

Animal surgery requires access to an animal care facility, and must comply with the established guidelines for ethical conduct in the care and use of animals in research (www.iacuc.org). Compared to humans, the smaller size of the rat vas deferens and epididymis can be challenging to work with. However, this is the only model that allows for the practice of vasoepididymostomy techniques, and for the assessment of long-term anastomotic patency.

Six to eight week-old male Sprague-Dawley rats (200-300 g) are ideal for male infertility microsurgical training and research. In these rats, occlusion of the vas deferens with two small metal clips, without division of the vas deferens, results in maximal epididymal tubule dilatation approximately seven days later (12). The rat vas deferens measures approximately 1.5 to 2 mm in outer diameter, and 0.15 to 0.25 mm in luminal diameter, compared to the 2 mm outer diameter and 0.5 mm luminal diameter for the human vas. Vasal occlusion, as described above, can achieve dilation of the testicular end of the vas deferens to a luminal diameter of 0.5 mm, mimicking the discrepant luminal diameters seen in vasectomized patients seeking reversal. For the advanced trainee, the unobstructed rat model may also be used for microsurgical training, which is considerably more demanding in terms of technical perfection.

Anesthesia for rat microsurgery surgery should be administered in keeping with institutional guidelines. In the author's experience, intraperitoneal injection of xylazine (10 mg/kg) mixed with ketamine chloride (100 mg/kg), and re-dosed as needed during the procedure, provides sufficient anesthesia and analgesia.

Rat vasovasostomy is performed as an end-to-end anastomosis using either a one-layer or two-layer technique, as described previously for silicone tubing and human vasal segments. Hemostasis during infertility surgery in the animal model, as well as the operating room setting, should always be achieved with bipolar cautery, which creates a much smaller area of tissue damage than monopolar cautery.

Vasoepididymostomy represents the most challenging procedure in male infertility microsurgery. The success of this operation is heavily dependent on the quality and extent of surgical training and practice in the laboratory setting. Over the years, several different techniques have been described for vasoepididymostomy (13). This review discusses the longitudinal, end-to-side, two-suture intussusception technique (14).

Animals should be prepared for vasoepididymostomy by vasal occlusion, as described above. Following anesthesia induction, a midline incision allows easy access to the

reproductive organs. The gubernaculum is divided to allow complete mobilization of the testis and epididymis. The epididymis is gently separated from the testis, and the area of maximal epididymal tubular dilation is identified. A window is created in the tunic overlying this area, and the epididymal tubule of interested is identified. An anchoring stitch of 9-0 or 10-0 single armed suture is placed between the tunic and the vasal adventitia in order to decrease the pressure on the anastomosis. Using double-armed 10-0 nylon sutures, one needle from each of the two sutures is then placed longitudinally along both edges of the selected epididymal tubule, in a parallel fashion, without pulling through. A microknife or fine microscissors are used to open the epididymal tubule between the needles. Following this, the needles are pulled through the epididymal wall and prepared for placement through the vas. The needles are passed inside out from the vasal mucosa to the muscularis at the 2, 4, 8 and 10 o'clock positions. These positions should be pre-marked on the vas using the microdot marker. The sutures are then securely tied down, allowing the epididymal tubule to be intussuscepted within the lumen of the vas. Single armed 9-0 or 10-0 suture is then used to place additional interrupted stitches between the adventitia of the vas deferens and the tunic surrounding the epididymal tubule, in order to achieve a water-tight anastomosis.

The use of single-armed suture for vasoepididymostomy in the rat model has been previously described (5). This technique is best mastered in a controlled laboratory setting, but has wide applications in situations where double-armed suture is either unavailable or prohibitively expensive. Mechanical patency across the anastomosis following either technique can be demonstrated in the laboratory using a retrograde injection of indigo carmine into the vasal lumen, using a 27-guage angiocatheter sheath.

Evaluation of surgical skills

Objective evaluation of skills developed in the microsurgical training laboratory is important for documenting the trainee's progress as well as identifying areas that require further practice. Different methods of evaluating the technical skills gained by novice microsurgeons in the laboratory have been reported, ranging from direct observation and assessment by experts to the ability of surgeons to complete timed surgical drill tasks (1,3,15). The author recommends using a detailed and systematic trainee evaluation checklist, which objectively evaluates each of the steps involved in a microsurgical anastomosis, including tissue manipulation, suture handling, tying secure and square knots, correctly setting-up the anastomosis, and progressing through the procedure in a methodical fashion.

Summary

Laboratory-based practice to enhance microsurgical skills improves the surgeon's confidence, and reduces stress and operating time, benefiting both the patient and the surgeon. Because success in male infertility surgery is so heavily dependent on surgical skills, and because patient outcomes are usually not apparent until weeks or months following surgery, it is imperative that cognitive and technical competency in microsurgery be achieved before entering the operating room. Establishing a microsurgical laboratory need not be expensive, and does not require a lot of elaborate equipment. The availability of such a facility enables advancement of surgical skills, and allows for research and experimentation, which, in turn, encourages innovation and advancement of the field of male infertility microsurgery.

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Footnote

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