# Sperm Extraction in Obstructive Azoospermia

## What's Next?

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**KEYWORDS**

• Sperm retrieval • Obstructive azoospermia • Infertility

**KEY POINTS**

- The evolution of operative techniques for sperm retrieval, coupled with the introduction of in vitro fertilization and intracytoplasmic sperm injection, have afforded previously untreatable men with obstructive azoospermia reliable pathways to conception.
- Percutaneous sperm aspiration techniques have remained highly effective tools with minimal modifications since their introduction.
- Open approaches to sperm extraction continue to shift toward more minimally invasive practices in the hopes of facilitating their use in the clinic setting while minimizing patient morbidity.
- Innovations in sperm selection and purification may offer a means of improving the fertility potential of specimens and address important sperm parameters, including DNA fragmentation.

## INTRODUCTION

Advancements in operative technology, coupled with the introduction of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), have afforded previously untreatable infertile men with reliable pathways to conception. In particular, the introduction of the surgical microscope revolutionized the surgical management of male infertility and sperm retrieval. For men with obstructive azoospermia (OA), sperm can now be extracted from several different sites using a variety of surgical techniques. The obstruction can occur anywhere along the passage of sperm from the efferent ducts within the testis, along the epididymis, through the vas deferens, the ejaculatory ducts, the penile urethra, or even the urethral meatus. Of the 15% of infertile men presenting with azoospermia, approximately 30% to 40% have an obstructive cause.[1,2] Because of preserved spermatogenesis, sperm extraction with high-quality samples can be obtained upstream from the site of obstruction or by relieving the obstruction itself. This extraction is accomplished through reconstructive microsurgery, resection of the obstruction, percutaneous aspiration, or open surgical retrieval.

Although the last few decades have produced reliable surgical options for men with OA, further advances show promise in improving outcomes, reducing surgical time, and decreasing procedure-related morbidity. This article traces the evolution of sperm extraction techniques for OA and highlights new developments and innovations in sperm selection and purification.

## HISTORY AND EVOLUTION OF SPERM EXTRACTION TECHNIQUES

The first reported use of aspirated sperm was published by Temple-Smith and colleagues[3] in 1985. The case involved a 42-year-old man with a history

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of vasectomy and 2 subsequent failed reversals with vasoepididymostomy. Following prolonged epididymal massage and aspiration, a total of 0.2 mL was retrieved with 76% motility and an estimated concentration of $4.28 \times 10^6$ sperm per milliliter. Successful fertilization and clinical pregnancy was achieved through IVF using this sample. Building on this work, Silber and colleagues[4] published their approach to microsurgical epididymal sperm aspiration (MESA) in 1988. The article outlines a technique for epididymal sperm aspiration under 10 to 40 times magnification that begins in the distal corpus of the epididymis and continues proximally until motile sperm are retrieved. The 2 patients in whom this procedure was initially described both had congenital bilateral absence of the vas deferens (CBAVD). This new technique to be used in conjunction with IVF was well received and offered a path to pregnancy for men with OA. However, early fertilization and pregnancy rates did not produce favorable results. Many centers reported a success rate less than 10%.[5] Poor fertilization and pregnancy rates, coupled with the need for an operative microscope, limited the initial uptake of the MESA approach. The advent of ICSI a few years later led to significant improvements in outcomes with epididymal sperm.[5] With these changes came renewed interest in MESA. More recently, modifications to the MESA technique have been published, including the mini-MESA, obliterative MESA, and minimally invasive epididymal sperm aspiration (MIESA).[6–8]

Nearly 10 years after Temple-Smith and colleagues[3] published their technique of epididymal sperm aspiration, Craft and colleagues[9] described a percutaneous approach using a 21-gauge needle. This approach formed the basis of what is now considered a conventional percutaneous epididymal sperm aspiration (PESA). The procedure was well received because many surgeons did not have access to an operating microscope to perform MESA. PESA was initially performed with intravenous or general anesthesia but is now commonly done with local anesthesia in the office setting.[7]

The introduction of ICSI in 1992 made it possible to use sperm aspirated from the testes.[10] The first uses of testicular sperm for fertilization were reported in 1993 by Schoysman and colleagues.[11] They describe obtaining samples by testicular biopsy in men who were previously unable to produce an epididymal sperm sample. This technique is now commonly referred to as testicular sperm extraction (TESE). Using ICSI, successful fertilization and pregnancy was achieved.[11] This method overcame initial concerns of the fertilizing potential of less mature testicular sperm. In an attempt to minimize morbidity, percutaneous testicular sperm aspiration (TESA) was explored. Before this, TESA had been described as a diagnostic tool in azoospermic men.[12] The first report of TESA for ICSI was published by Bourne and colleagues[13] in 1995. Their technique used a 20-gauge Menghini biopsy needle under negative pressure in 2 men with OA. High rates of normal fertilization and subsequent pregnancy were achieved using the aspirated sample.[13] TESA was seen as a way to overcome the need for an operative microscope, avoid general anesthesia, and reduce patient morbidity. The procedure has evolved over time to include multiple needle passes with thinner-gauge needles.[12–15] The most recent development in sperm retrieval from the testis is microdissection testicular sperm extraction as first described by Peter Schlegel[16] in 1999. After observing that seminiferous tubules had different morphologic characteristics under the operating microscope, selective extraction of larger tubules (more likely to contain sperm) was performed. This technique allowed improved identification and retrieval of sperm while removing less tissue from the testis. For men with nonobstructive azoospermia (NOA), the technique has emerged as a more effective and reliable technique than multiple-pass TESE.[10]

Given the success of TESA and PESA percutaneous approaches, Qiu and colleagues[17] explored vasal sperm aspiration as another means of obtaining sperm percutaneously. Their 1997 article discussed percutaneous vasal sperm aspiration (PVSA) in 6 men diagnosed with ejaculatory duct obstruction. Of the 6 men included in the study, adequate sperm for intrauterine insemination (IUI) was obtained in 3 men. Only 1 resulted in a pregnancy. With the vas deferens fixed to the skin by a clip, a 21-gauge needle was advanced into the lumen of the vas deferens followed by a 23-gauge blunt needle. The 23-gauge needle was advanced through the 21-gauge needle in the direction of the epididymis. Aspiration was done using a 5-mL syringe.[17] The evolution of sperm retrieval techniques is shown in **Fig. 1**.

## CURRENT ROLE OF EPIDIDYMAL AND TESTICULAR SPERM RETRIEVAL IN OBSTRUCTIVE AZOOSPERMIA

### *Percutaneous Approaches to Sperm Retrieval*

Percutaneous methods of sperm retrieval provide several benefits to both patients and surgeons. These procedures are particularly appealing because they can be performed on short notice under local anesthesia in the outpatient setting,

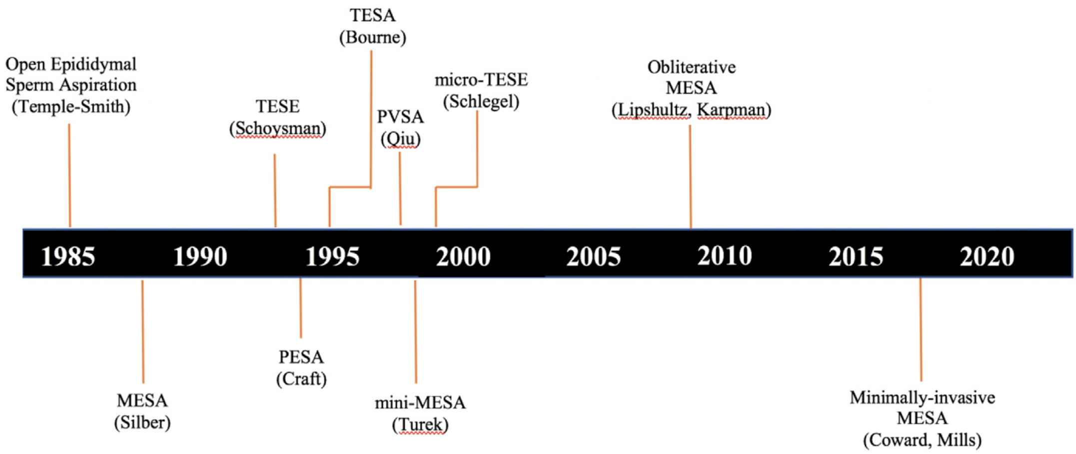


**Fig. 1.** Timeline of sperm retrieval techniques.

have minimal patient downtime, and are highly reproducible. Unlike more invasive methods of sperm retrieval, percutaneous aspiration does not require additional equipment or training in microsurgery. Percutaneous sperm extraction can be targeted at the level of the testis, epididymis, or vas deferens.

### Percutaneous Epididymal Sperm Aspiration

Among men with OA, sperm retrieval rates with PESA range from 51% to 100%, irrespective of the cause of their obstruction.[7] Retrieval of motile sperm is high, with reported rates ranging from 62% to 94%.[18] In men with postvasectomy OA who do not desire a reversal, PESA offers an appealing method of sperm extraction. Collins and colleagues[19] reported one of the few comparative studies with PESA as an intervention. They performed MESA and PESA on both testes in men with previously proven fertility seeking vasectomy reversal. There was no difference in the rate of successful sperm retrieval between MESA and PESA. These investigators therefore advocate PESA when possible in men with OA secondary to vasectomy. More recently, Yafi and Zini[18] reported on 255 men with OA undergoing PESA. The study included men with OA of various causes, including vasectomy, vasectomy with prior failed reversal, and CBAVD. Motile sperm was found in 75.3% of men. Younger paternal age and testicular size were predictive of finding motile sperm. For patients with a prior history of PESA, repeat PESA has been reported on the ipsilateral testis with lower rates of sperm retrieval (26.3%).[20] One important consideration with PESA is that up to 25% of patients are unsuccessful in retrieval of sperm on their first attempt.[18] Patients then require a subsequent TESA or TESE. The rate of complications in PESA has been reported at 3.4% and includes pain, hydrocele, infection, and swelling.[21]

### Testicular Sperm Aspiration

Retrieval of testicular sperm by percutaneous needle aspiration can be done in the outpatient setting with reliable results. TESA is most commonly performed on the day of egg retrieval because the amount of testicular tissue is minimal and may not be adequate for cryopreservation. However, Garg and colleagues[22] reported TESA outcomes in a retrospective case series of 40 patients from 2003 to 2007 and had adequate sperm retrieved for cryopreservation in 39 of 40 patients (97.5%) with no complications reported. In the modern-day evaluation of OA, TESA has continued utility as a diagnostic procedure. Among men with indeterminate clinical findings for OA versus NOA, it can be used to determine the presence or absence of spermatogenesis. There is also a role for TESA in the setting of a failed PESA. Often now termed a rescue TESA, this approach has been shown to have higher rates of successful sperm retrieval than PESA and represents an alternative backup option when PESA is unsuccessful. The quantity and motility of sperm in these cases tends to be lower than in a successful PESA.[7] Although TESA with proper technique results in rates of sperm recovery sufficient for ICSI in nearly 100% of men with azoospermia, other methods of sperm aspiration may produce superior samples with quantity more sufficient for cryopreservation.[23]

### Percutaneous Vasal Sperm Aspiration

Vasal sperm aspiration is an option for men with obstruction at the level of the prostate or distal vas deferens, as well as in men with ejaculatory dysfunction. Reports of PVSA to achieve pregnancy have shown the technique to be highly successful. Qiu and colleagues[24] published their series of 26 patients with anejaculation who underwent sperm retrieval with PVSA followed by IUI. There was a 100% retrieval rate, with a pregnancy rate of 73.1%. Sperm was retrieved in sufficient volume and quality for IUI. Vasal sperm have the benefit of full maturation, making them an excellent sample for subsequent ICSI, IVF, IUI, or cryopreservation.[25] The site of obstruction is an important factor when PVSA is being considered, because healthy sperm in the scrotal vas are only likely to be present in cases of more distal obstruction, such as inguinal or ejaculatory duct obstruction.

## Open Surgical Approaches to Sperm Retrieval

Although more invasive than percutaneous approaches, open surgical sperm extraction techniques play an important role in the diagnosis and management of men with OA. Both TESE and MESA reliably produce large numbers of sperm in men with OA.

## Testicular Sperm Extraction

In men with OA, there is no consensus with respect to the superiority of sperm retrieved from the epididymis or testis in terms of IVF/ICSI outcomes, assuming sperm are successfully retrieved and readily available for use by the embryologist. Despite promising results of early studies of epididymal sperm, systematic reviews and meta-analyses have failed to find sufficient evidence to recommend one sperm retrieval technique rather than another.[26–28] Over time, TESE has become the most well-known and ubiquitous sperm retrieval technique, in large part because of the familiarity of urologists with testicular biopsy.

In men with OA, TESE produces a near-100% sperm retrieval rate. TESE has an important diagnostic role in men with normal testicular volume, palpable vasa deferentia, and normal or near-normal serum follicle-stimulating hormone levels. In addition to providing a tissue diagnosis of OA for men with no sperm in their samples, TESE allows extraction of a sufficient volume of sperm for cryopreservation. Any other method of percutaneous or open sperm retrieval that fails to identify sperm may be converted to a TESE with relative ease, and the ability to maneuver the conversion to a TESE should be made feasible within the chosen operative setting.

## Microsurgical Epididymal Sperm Aspiration

MESA offers several benefits as a method of sperm retrieval in men with OA. Retrieval rates in appropriately selected men approach 100%. The number of sperm retrieved far exceeds those required for a single ICSI/IVF cycle and the sperm can be cryopreserved in 98% to 100% of cases. On average, MESA yields $15 \times 10^6$ to $95 \times 10^6$ total sperm with 15% to 42% total motility.[29,30] Combined with ICSI, epididymal sperm obtained by MESA has a clinical pregnancy rate of 42% to 60%.[28,30,31] Unlike TESE and percutaneous retrieval methods, MESA requires the use of an operative microscope and additional microsurgical training, which may limit its use by practitioners who either do not have access to a microscope or are less familiar with microsurgical techniques.

## Minimally Invasive Epididymal Sperm Aspiration

Although MESA has emerged as reliable sperm retrieval procedure for men with OA, advances in technical aspects of the procedure have been designed to reduce the morbidity and complexity of the procedure. The mini-MESA, first described in 1998, decreased the incision size on a traditional MESA in hopes of improving postoperative pain and recovery time.[6,8] However, this did not address one of the main factors limiting the clinical use of MESA: the need for an operative microscope. Coward and Mills[7] further simplified the mini-MESA by performing the procedure solely under loupe magnification without compromising sperm yields. This approach is called a MIESA and can be performed either under oral or monitored anesthesia care (MAC) sedation.[7]

A MIESA begins much in the same way as a mini-MESA with a 1-cm transverse upper hemiscrotal incision. The testicle is exposed and an eyelid retractor is positioned within the tunica vaginalis to maintain exposure (Fig. 2). The caput of the epididymis is then rotated into the window opening and a 3-0 traction suture is placed in the upper third of the epididymis (Fig. 3).

The head of the epididymis is then gripped with the surgeon's nondominant hand as the assistant prepares a 1.0-mL tuberculin syringe with a 24-gauge angiocatheter tip primed with 0.1 mL of sperm wash medium. Additional syringes are prepared in similar fashion to allow smooth transition from one syringe to another. A 15° double-beveled straight ophthalmic blade is then passed into the epididymis in a single motion (Fig. 4). As the blade is slowly withdrawn, the epididymis is compressed and the assistant aspirates the expressed epididymal fluid. A single drop of the aspirate is evaluated in real time by a certified laboratory andrologist to confirm the presence of motile

Fig. 2. Testis exposure using an eyelid retractor and mosquito forceps to facilitate closure.

sperm. If sperm are not immediately identified, progressive epididymotomies can be made proximally until high-quality motile sperm are extracted. Once high-quality sperm are identified, all proximal tubules are aspirated for cryopreservation.

## FUTURE DIRECTIONS

The technical aspects of sperm retrieval have been honed throughout the years. Sperm samples sufficient for IVF or ICSI can now be reliably obtained from the epididymis or testicle via a variety of approaches, as described in this article. With continued success with sperm extraction and achievement of live birth via IVF/ICSI there has been an increasing focus on determining which sperm characteristics will lead to the best pregnancy and functional outcomes for offspring.

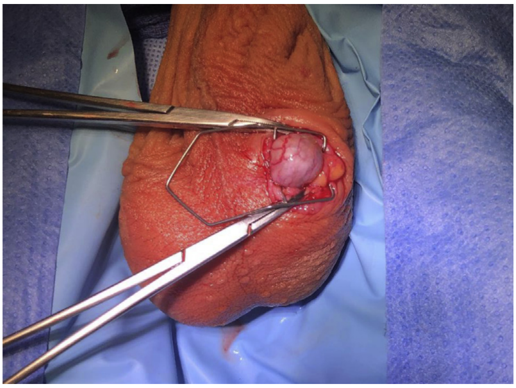

Fig. 3. Epididymal exposure.

Pregnancy outcomes between extraction sites have been examined in multiple studies with varied results. A meta-analysis of comparative studies in 2004 found there was no difference in IVF/ICSI outcomes between epididymal and testicular sperm.[32] A study in Denmark approached the questions of gamete source location from a developmental standpoint and compared functional outcomes of children born via IVF/ICSI using epididymal versus ejaculated sperm. Children born from epididymal sperm had equivalent motor skills, language skills, and rates of malformation compared with children born with ejaculated sperm.[32] This finding is in contrast with a 2012 *New England Journal of Medicine* article that found that children born via ICSI may be at higher risk for birth defects compared with children born naturally or even via conventional IVF.[33]

The theory behind the possibly increased risk of birth defects with ICSI is that, by performing ICSI, many of the intrinsic sperm selection processes are bypassed. In response to this concern, many of the emerging research studies and technologies are focused on sperm selection. Going beyond the traditional selection techniques used for ejaculated sperm, such as density-gradient centrifugation, sperm washing, and swim-up test, these emerging technologies include the role of DNA fragmentation as well as sperm selection with microfluidics and nanotechnology.

### *DNA Fragmentation*

High rates of sperm DNA fragmentation are associated with worse outcomes in natural conception and IUI.[34] With respect to the impact of DNA fragmentation in IVF and ICSI, the data are more heterogeneous. Nevertheless, recent meta-analyses of the impact of high levels of DNA fragmentation on IVF outcomes have confirmed a negative effect. Zini[35] published a review of 11 studies and found a combined odds ratio (OR) of 1.70 (confidence interval [CI], 1.30–2.23) correlating high DNA fragmentation and failure to achieve pregnancy. An update to this review was published in 2017 with the addition of 9 additional articles. Again, higher levels of DNA fragmentation correlated with failure to achieve pregnancy (OR, 1.65; CI, 1.34–2.04).[36] The same meta-analysis also examined the effect of sperm DNA fragmentation on ICSI outcomes. Data combined from 24 studies found an OR of 1.31 (CI, 1.08–1.59) for ICSI failure among men with higher levels of DNA fragmentation.[36] Not all meta-analyses have confirmed the association of high DNA fragmentation and worse ICSI/IVF

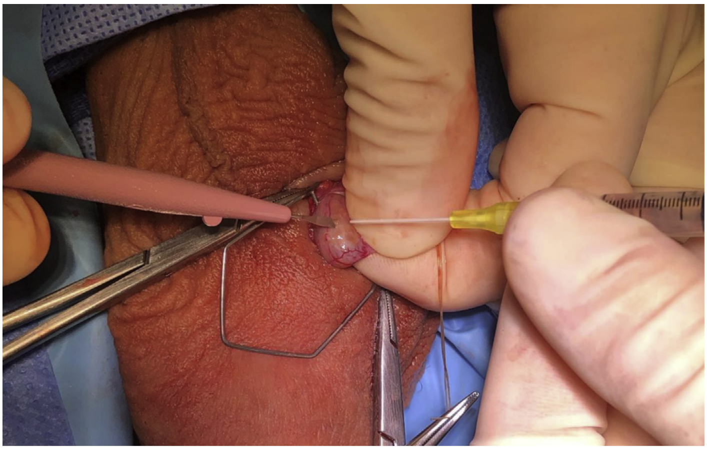

**Fig. 4.** Epididymotomy with ophthalmic blade followed by aspiration of epididymal fluid using 24-gauge angiocatheter on a syringe primed with sperm wash medium.

outcomes. Although Zhang and colleagues[37] found an association between DNA fragmentation greater than 27% and worse IVF outcomes, this did not hold true when studies were controlled for the type of fragmentation test used. More important than pregnancy rates as an outcome for IVF and ICSI are live-birth rates. Osman and colleagues[38] completed a systematic review and meta-analysis of live-birth rates with IVF or ICSI using sperm with high levels of DNA fragmentation. Greater fragmentation was associated with lower live-birth rates. Despite significant heterogeneity in individual studies, the predominant conclusion from meta-analyses and systematic reviews is that of an association between higher rates of sperm DNA fragmentation and poor outcomes with IVF and ICSI.[36]

Selection of testicular sperm may provide a means of reducing DNA fragmentation levels. In men with high levels of fragmentation in ejaculated samples, sperm retrieved directly from the testis has been shown to have lower levels of DNA fragmentation and better outcomes with ICSI.[21,39] Moskovtsev and colleagues[40] examined levels of DNA fragmentation in men with persistently high fragmentation following a 12-month course of oral antioxidants. Rates of DNA fragmentation were 3 times higher in ejaculated sperm compared with testicular sperm. A small series of men with OA found similar results. The study noted that DNA fragmentation rates were nearly twice as high in epididymal spermatozoa independent of the cause of OA.[41] There is some evidence of improved ICSI outcomes using testicular sperm in men with high levels of DNA fragmentation.[39] To date, only 1 prospective study has been published investigating treatment outcomes between ejaculated and testicular sperm. Esteves and colleagues[42] followed 172 men with high levels of DNA fragmentation undergoing ICSI. For the testicular sperm group, the relative risk for miscarriage was 0.29 (CI, 0.10–0.82) and the relative risk for live birth was 1.76 (CI, 1.15–2.70).

## *Emerging Techniques in Sperm Selection*

Within a single sample, there is great heterogeneity with respect to the quality of individual sperm.[43] Since the introduction of ICSI in 1993, several techniques have been adopted to identify and select those sperm with the greatest fertilizing potential. These techniques range from conventional procedures such as sperm swim-up, glass wool filtration, and density-gradient centrifugation to more advanced techniques such as sperm magnetic sorting and high-magnification microscopy.[34,44] Microfluidics and nanotechnology are two emerging techniques with the potential to isolate good-quality sperm with a greater degree of precision.[45,46]

### *Microfluidics technology in sperm selection*

The study of microfluidics involves the use of submillimeter channels to manipulate small volumes of fluid. A microchip is then able to select out various components of the fluid.[47] When run through a microfluidic device, healthy sperm is selected out into the chip from the channel. In general, there are 3 categories of microfluidic devices for sperm selection and sorting: those that isolate based on motility alone, those used for the observation and selection of individual sperm, and those that select sperm based on factors other than motility.[45] The use of microfluidic technology in sperm processing has been shown to produce samples with lower levels of DNA fragmentation and reactive oxygen species, and better motility.[48] Quinn and colleagues[49] compared rates of DNA fragmentation in sperm samples processed by microfluidic chip with those sorted through density-gradient centrifugation. Median DNA fragmentation index was 21% in the unprocessed semen sample, 6% in density-gradient centrifugation, and 0% by microfluidic chip. In a retrospective cohort study of couples undergoing IUI for infertility, microfluid sperm sorting resulted in higher ongoing pregnancy rates (15.03%) compared with density-gradient processed samples (9.09%). The OR of an ongoing pregnancy in the microfluidic group was 3.49 (CI, 1.12–10.89).[50] Further prospective, randomized trials are needed to assess the full extent and potential benefit of sperm selection with microfluidic technology.

### *Nanotechnology for sperm selection*

Nanotechnology (the use of 1–100-nm materials with specific biological or chemical properties) has shown promise in sperm selection and labeling.[46] The field has expanded rapidly in biomedicine and now shows great potential in reproductive medicine.[51–53] Nanoparticles have the ability to remove less favorable sperm from a sample through a process termed nanopurification. For example, magnetic iron oxide nanoparticles have been shown to clear large semen volumes of acrosome-reacted or apoptotic spermatozoa.[46,54] This form of nanopurification has shown similar effects to established magnetic-assisted cell-sorting technologies.[46] Human studies examining the impact of nanopurification on fertility rates have not yet been completed. Nanotechnology has also been applied as a diagnostic tool for male infertility. Vidya and Saji[55] tethered heparin onto gold nanoparticles as a way to detect protamine levels in semen samples. As the most abundant nuclear protein in human sperm, protamine levels play an important role in the morphology of sperm,[56] which allows it to be used as a targeted biomarker to evaluate the fertility potential of a given semen sample. The binding of protamine to the heparin-tethered nanoparticles induces a color change to the naked eye that could be easily interpreted.[55] Although nanotechnology shows great potential in reproductive medicine, the human application of many of the nanoparticles in development has yet to be assessed.

## SUMMARY

Men with OA have the benefit of a wide array of sperm extraction techniques that cater to the cause of their obstruction and produce reliable results in the hands of male fertility specialists. Percutaneous sperm aspiration techniques have remained highly effective tools with minimal modifications since their introduction. Open approaches to sperm extraction continue to shift toward less invasive practices in the hopes of facilitating their use in the clinic setting while minimizing patient morbidity. Innovations in sperm selection and purification may offer a means of improving the fertility potential of surgically retrieved specimens and address important emerging sperm parameters, including DNA fragmentation.

## DISCLOSURE

The authors have nothing to disclose.

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