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Surgical Sperm Retrieval Techniques for Assisted Reproductive Technology

Yardımcı Üreme Teknolojisi için Cerrahi Sperm Elde Etme Teknikleri

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ABSTRACT

Intracytoplasmic sperm injection provides in vitro fertilization for many infertility conditions. It is also an important treatment option for fertilization in azoospermic men. Sperm production in azoospermic male testicles is possible. In these patients, sperm extraction from the epididymis or testis is required before intracytoplasmic sperm injection. Numerous sperm retrieval procedures for obtaining sperm from the epididymis or testicles have been developed. The spermatozoa obtained by these methods are used in intracytoplasmic sperm injection for in vitro fertilization. The most important factor when determining the sperm retrieval method is whether the azoospermia is obstructive or non-obstructive. In addition, the experience of the surgeon performing the procedure is also effective in determining the sperm retrieval technique. In this review, sperm retrieval methods are presented, and current developments in these sperm retrieval methods are also mentioned. The indications of surgical sperm retrieval methods in both obstructive and non-obstructive azoospermic patients, the technical aspects of each method, possible complications, advantages and disadvantages of these methods are presented, and their superiority to each other are also discussed. In addition, evidence for the fertilization rates after intracytoplasmic sperm injection using sperm obtained by these methods and continuation of pregnancy is presented in a comparative manner and critically discussed.
Keywords: Sperm retrieval; assisted reproductive techniques; male infertility; azoospermia.

ÖZ

İntrasitoplazmik sperm enjeksiyonu, birçok infertilite koşulu için in vitro fertilizasyon sağlamaktadır. Azospermik erkeklerde de fertilizasyon için önemli bir tedavi seçeneğidir. Azospermik erkek testislerinde sperm üretimi mümkündür. Bu hastalarda intrasitoplazmik sperm enjeksiyonundan önce epididim veya testisten sperm alınması gerekir. Epididim veya testislerden sperm elde etmek için çok sayıda sperm alma prosedürü geliştirilmiştir. Bu yöntemlerle elde edilen spermler, in vitro fertilizasyon için intrasitoplazmik sperm enjeksiyonunda kullanılmaktadır. Sperm alma yöntemini belirlerken en önemli faktör azosperminin obstrüktif veya non-obstrüktif olup olmadığıdır. Ayrıca işlemi yapan cerrahın tecrübesi de sperm alma tekniğinin belirlenmesinde etkilidir. Bu derlemede sperm elde etme yöntemleri anlatılmış ve ayrıca bu sperm elde etme yöntemleri ile ilgili güncel gelişmeler de ele alınmıştır. Hem obstrüktif hem de non-obstrüktif azospermik hastalarda cerrahi sperm elde etme yöntemlerinin endikasyonları, her bir yöntemin teknik yönleri, olası komplikasyonları, bu yöntemlerin avantaj ve dezavantajları anlatılmış ve birbirlerine göre üstünlükleri de tartışılmıştır. Ayrıca, bu yöntemlerle elde edilmiş olan sperm kullanılan intrasitoplazmik sperm enjeksiyonu sonrası fertilizasyon oranları ve gebeliğin devamına ilişkin kanıtlar karşılaştırmalı bir şekilde sunulmuş ve eleştirel bir şekilde tartışılmıştır.
Anahtar kelimeler: Sperm toplama; yardımcı üreme teknikleri; erkek infertilitesi; azospermi.

INTRODUCTION

Two significant discoveries in male infertility have occurred recently (1-3). The first was the definition of intracytoplasmic sperm injection (ICSI) in males with significant spermiogram abnormalities for in vitro fertilization (1). The second was proof of ICSI can be used in azoospermic cases and that sperm from the epididymis or testicles can be used for normal fertilization and pregnancy (2,3). Azoospermia is the absence of spermatozoa in the ejaculate, which affects 1-3 percent of the male population. This percentage is around 10% among infertile males. Although azoospermia is the medical term for infertility, sperm production in azoospermic male testicles is possible (4). As a result, numerous sperm retrieval procedures for obtaining sperm from the epididymis or testicles have been developed. The spermatozoa obtained by these methods are used in ICSI for in vitro fertilization (1-6).

The most important factor when determining the sperm retrieval method is whether the azoospermia is obstructive or nonobstructive. In addition, the experience of the surgeon performing the procedure is also effective in determining the sperm retrieval technique. In obstructive azoospermia (OA), there is no defect in sperm production of the testicles. The produced sperm cannot be ejaculated to the obstruction of the seminal ducts (4,7). OA can occur for many reasons. It is defined as congenital or acquired. In rare cases of acquired OA such as vasectomy, there is a chance of normal fertilization by treating the obstruction with surgical methods (8). Although it is an effective treatment, recanalization may not be possible in some cases. In the majority of congenital or acquired OA cases, sperm can be obtained from the epididymis or testis.

Non-obstructive azoospermia (NOA) occurs due to defects in sperm production (4). NOA can also occur due to congenital and acquired causes. In NOA cases, the only method to obtain sperm before ICSI is to search for sperm in the testis. There is a possibility of spermatogenesis in different areas of the testis in men with NOA. Therefore, spermatozoa can be found in the testicles in 30-60% of cases (6). Testicular sperm extraction (TESE) is a sperm retrieval technique applied in NOA (6,9), and the sperm retrieval rate increases if microsurgery is applied (6,10). Table 1 summarizes the advantages and disadvantages of various sperm retrieval techniques.

PERCUTANEOUS SPERM RETRIEVAL METHODS

Percutaneous sperm retrieval methods are minimally invasive procedures. Its important advantages are that it can be applied in a short time under local anesthesia and can be repeated. It is easier to learn and less costly than surgical methods. The procedure can be performed by aspirating sperm from the testis, epididymis, or vas deferens (11).

Percutaneous Epididymal Sperm Aspiration (PESA)

The success rate of sperm retrieval in patients who underwent percutaneous epididymal sperm aspiration (PESA) for OA is around 51-100% (12). It has been reported that the motility of sperm obtained with PESA is between 62% and 94% (13). PESA is a useful sperm retrieval method in men with OA due to vasectomy. Collins et al. (14) reported a study comparing the success of sperm retrieval. They investigated men after vasectomy who were known to have no previous infertility problems. They performed microsurgical epididymal sperm aspiration (MESA) and

Table 1. Advantages and disadvantages of different sperm retrieval techniques

	Advantages	Disadvantages
PESA	Quick and inexpensive There's no need for microsurgical experience Repeatable minimal morbidity There will be no open surgery Instruments and materials are limited	Few sperm retrieved Fibrosis and obstruction at the aspiration site Risk of hematoma/spermatocele Limited number of sperm for cryopreservation
MESA	Large number of sperm retrieved High number of sperm for cryopreservation Reduced risk of hematoma Reconstruction possible[1]	Open surgical exploration required Increased cost and time-demanding Operating microscope required Microsurgical instruments and expertise required Postoperative discomfort
TESA	Fast and low cost; Repeatable No open surgical exploration No microsurgical expertise required Few instruments and materials Minimal/mild postoperative discomfort	Relatively low success rate in NOA cases Few sperm retrieved in NOA cases Limited number of sperm for cryopreservation Risk of hematoma/testicular atrophy
TESE	No microsurgical expertise required Repeatable	Costlier and more time-consuming Open surgical exploration is needed In NOA cases, few sperm are retrieved Risk of testicular atrophy[3] Risk of testicular androgen production may be impaired[3] Postoperative discomfort
Micro-TESE	Higher success rates in NOA cases[2] Larger number of sperm retrieved[2] Low risk of complications Relatively higher chance of sperm cryopreservation[2]	Surgical exploration required Operating microscope required Increased cost and time-demanding Postoperative discomfort Microsurgical instruments and expertise required

PESA: percutaneous epididymal sperm aspiration, MESA: microsurgical epididymal sperm aspiration, TESA: testicular sperm aspiration; TESE: testicular sperm extraction; micro-TESE: microsurgical testicular sperm extraction, NOA: non-obstructive azoospermia, [1]: in cases of post-vasectomy obstructions, [2]: compared with TESA and TESE in NOA cases, [3]: multiple biopsy-TESE

PESA on both testicles. Both procedures yielded the same percentage of effective sperm retrieval. As a result of this study, it was emphasized that PESA should be performed in men with OA after vasectomy. In the study conducted by Yafi and Zini (13), 255 men with OA for different reasons who underwent PESA were investigated. In this study, OA cases with many causes such as vasectomy and congenital bilateral absence of the vas deferens (CBAVD) were investigated. It was reported that 75.3% of motile sperm were found with PESA. In addition, it was determined that the probability of finding motile sperm was increased in young men and those with high testicular volume. If PESA was repeated on the ipsilateral testis, lower sperm retrieval rates (26.3%) were observed (15). It has been reported that 25% cannot find sperm with PESA (13). Therefore, additional procedures such as testicular sperm aspiration (TESA) or TESE may be required.

Testicular Sperm Aspiration (TESA)

TESA can be done in a variety of ways. Percutaneous aspiration of the testicular parenchyma is performed by introducing a fine or large-diameter needle into the testis through the scrotal skin. During needle insertion, the main branches of the testicular artery should not be injured. The needle is frequently inserted at an oblique angle towards the medium and lower testicular poles, at the anterolateral or anteromedial part of the superior testicular pole. Vascular structures are least likely to be found in these regions. To avoid vascular injury, loupe magnification might be utilized. Negative pressure is established by pulling the syringe plunger to aspirate the sperm in the seminiferous tubules. Furthermore, the needle's tip is pushed in and out of the testis on an oblique plane to reach different areas. A tube holding a hot sperm medium is filled with the sample. The sperm is taken to a laboratory and examined under a microscope. In case of unsuccessful results, TESE or TESA can be performed on the contralateral testis (16).

Percutaneous Vasal Sperm Aspiration (PVSA)

Vasal sperm aspiration can be applied to infertile men who develop due to obstruction at the prostate or distal vas deferens level. It is also an option for men with ejaculatory dysfunction. Percutaneous vasal sperm aspiration (PVSA) is a successful technique with a high rate of achieving pregnancy. Qiu et al. (17) reported the results of sperm retrieval with PVSA. This study consisted of infertile men with anejaculation. There was a pregnancy rate of 73.1% and a 100% retrieval rate after intrauterine insemination (IUI). Sperm in the vas deference are mature and in high volume. Therefore, sperm obtained after PVSA are of high quality for assisted reproductive technology (18). PVSA localization should be determined according to the level of obstruction. In cases of distal obstruction such as inguinal or ejaculatory duct obstruction, the scrotal vas deferens may be preferred.

SURGICAL SPERM RETRIEVAL METHODS

Microsurgical techniques or conventional methods are used in surgical sperm retrieval. These procedures are more painful than percutaneous sperm retrieval techniques. Therefore, it can be performed under general anesthesia. Local anesthesia can be preferred with intravenous sedation or epidural anesthesia (16).

Microsurgical Epididymal Sperm Aspiration (MESA)

Temple-Smith et al. (19) described MESA for the first time and reported it in 1985. During the procedure, a 2-3 cm transverse incision is made into the scrotum. Its tunic opens and a large-looking tubule is found. The fluid leaking from the tube is aspirated and poured into a tube containing the hot medium. It is transferred to the laboratory through these tubes and the sample is examined. Sperm count or quality may not be sufficient. Re-aspiration is performed from a different part of the epididymis (from cauda to hood) and/or contralaterally. If motile sperm cannot be collected after the procedure is repeated, simultaneous TESE or TESA can be used (16).

Conventional Testicular Sperm Extraction (TESE)

Taking samples from the testicular parenchyma for sperm research and the use of found sperm in in vitro fertilization were first described by Devroey et al. (3) in 1995. It is a standard open surgery method and no optical magnification is performed. It can be performed under general anesthesia. Local anesthesia can be preferred with intravenous sedation or epidural anesthesia. The "window" method is used in the operation. An approximately 2 cm long transverse incision is made in the scrotal skin. If there is a possibility of sperm extraction from both testicles, a vertical incision can also be made from the scrotal raphe. The testis is reached by passing the subcutaneous layers, dartos, and tunica vaginalis. The testicular parenchyma is exposed by making an incision of approximately 1 cm on the tunica albuginea. On removing the testicular parenchyma, little pressure is given to the testicle. A small piece of testicular parenchyma is cut with scissors and a sample is taken. After that, the sample is placed in sperm culture media. Multiple samples can be taken from the same incision if necessary. Additional albugineal incisions may be made in the upper, middle, and lower testicular poles to obtain multiple biopsy specimens. Samples are sent to the laboratory for sperm analysis. After an adequate sample is taken, the non-absorbable suture closes the tunica albuginea (16).

Microsurgical Testicular Sperm Extraction (micro-TESE)

TESE with a microscope in azoospermic patients was first reported by Schlegel (20) in 1999. Delivery of testis is performed as described in MESA. A single, large, mid-section incision is then made under 6-8x magnification in an avascular region of the tunica albuginea, exposing the testicular parenchyma extensively. Large seminiferous tubules are searched with the microscope. Dissection of testicular parenchyma is performed with 16-25x magnification. Superficial and deep areas of the testicular parenchyma can be examined under the microscope. Multiple biopsies can be taken during this procedure. Enlarged tubules may not be seen during the procedure. In this case, any tubule that is different is excised. If all tubules look the same, random micro-biopsies are done from different areas. The sperm cells in the samples should not lose their vitality. Therefore, the tissues are transferred with a Petri dish containing the sperm medium. Blood clots are cleaned from the samples before they are examined. Tunica albuginea is closed with non-absorbable sutures. The process is terminated by closing the scrotal layers with absorbable sutures (16). The microsurgical testicular sperm extraction (micro-TESE) surgical method is shown step by step in Figure 1.

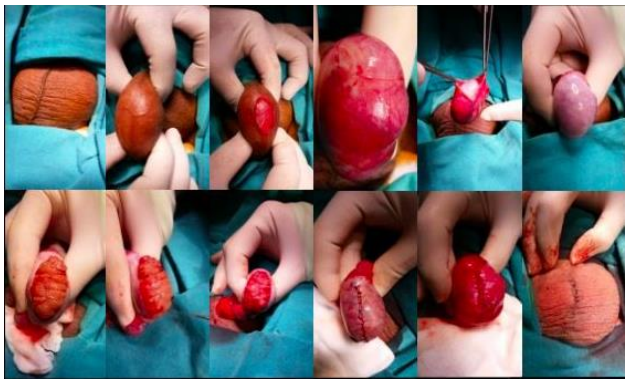

Figure 1. Microsurgical testicular sperm extraction surgical method (photos from Dr. Tahsin Turunç's personal archive)

Sperm Retrieval Postoperative Care and Complications

Percutaneous sperm retrieval methods are minimally invasive methods. The procedure is applied without hospitalization to the patient. Patients usually return to their normal activities the next day. Although open surgery (micro-surgery or conventional) is more invasive than percutaneous methods, they return to their normal activities after 2-3 days. Scrotal cold application and elevation are recommended in patients to reduce edema and relieve pain. For about a week, patients are recommended to avoid ejaculation and strenuous physical activity. Oral analgesics and anti-inflammatory drugs should be used for complaints of pain and scrotal swelling (6).

Complications such as persistent pain, infection, swelling, hematoma, and hydrocele may occur after sperm retrieval. The incidence of complications has been reported to vary between 0-70% (21-24). PESA complications generally have minimal morbidity compared to open surgery. Complication rates vary according to the type of procedure. Rarely, it varies according to the type of azoospermia. Fibrosis at the aspiration site is common in percutaneous methods, but serious complications are rare (16). Intratesticular hematoma often occurs in patients undergoing TESE due to single or multiple biopsies. However, it usually resorbs spontaneously without damaging the testicular function (23). Devascularization may develop in the testicular parenchyma after conventional TESE. Therefore, a temporary or permanent decrease in serum testosterone level may be observed (9,22). The complication rate is higher in conventional TESE compared to micro-TESE (9,20,22,24). During micro-TESE, before the tunica albuginea incision is made, the underlying testicular vessels are detected. Thus, intratesticular blood flow is preserved. It also causes minimal parenchymal damage by identifying tubules that are likely to produce sperm (22). Since androgen production is low in Klinefelter syndrome (KS) patients, a decrease in serum testosterone has been reported after micro-TESE (21). However, in most KS patients, testosterone levels return to preoperative values during the 1-year follow-up period. Sperm retrieval methods should be performed by surgeons with adequate training to reduce the risk of postoperative complications (25).

Comparative Outcomes and Expectations

Cochrane review has been reported that randomized controlled studies comparing sperm retrieval techniques are not sufficient and that the simplest and least invasive technique should be chosen (26). There is a meta-analysis study comparing ICSI results (n=1,103 cycles) of sperm from men with OA and NOA. In this study, it was determined that the success of ICSI was lower in NOA cases. OA cases had higher natural fertilization rates (relative risk, RR: 1.18; 95% confidence interval, CI: 1.13-1.23) and clinical pregnancy rates (RR: 1.36; 95% CI: 1.10-1.69) when compared to NOA cases. In these groups, the rise in ongoing pregnancy rates was not significant (RR: 1.19; 95% CI: 0.87-1.61). There was no statistically significant difference in implantation (RR 1.01; 95% CI: 0.87-1.61) or abortus rates (RR: 0.84; 95% CI: 0.48-1.48) between the groups (27). The findings of this meta-analysis matched Cochrane's recommendations.

For the treatment to be successful, the laboratory effort and expectations around surgical sperm retrieval operations techniques are equally critical. Partners must be prepared for ICSI simultaneously time to enhance treatment success. In NOA, the experience of the andrology laboratory increases the success of the procedure. It is recommended to use the "recommended minimum search time" scale for adequate effort during sperm collection. Laboratory technicians are more equipped for the work at hand when it is considered that the effort is aligned with procedural complexity. Furthermore, since testicular sperm motility is often stable after incubation in vitro for at least 24 hours following retrieval, the use of testicular sperm obtained prior to ICSI is now regularly done to ease the procedure schedule for both couples (28).

CONCLUSION

In spite of the fact that there is a wide range of careful sperm retrieval procedures, there is no proof that it is an ideal method for both OA and NOA. Generally, sperm production may be insufficient or focal in NOA, and it may be very difficult to find sperm compared to OA. There are not any randomized controlled studies in the literature that are contrasting the viability of sperm retrieval strategies for NOA. The patient's clinical circumstance ought to be founded on, as every technique has its strengths and restrictions. Since ICSI has not generally success, urologists need to perform dependable techniques that have lesser morbidity and acquire adequate sperm for different ICSI techniques. With a superior comprehension of the cryobiological behavior of sperm and the overall reproductive capacity of sperm from anatomical physical sources, sperm retrieval procedures can be optimized and individualized.

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