Review Article

# Innovations in surgical management of nonobstructive azoospermia

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## ABSTRACT

Testicular sperm extraction (TESE) technique and intra-cytoplasmic sperm injection are breakthrough fertility treatments for men with nonobstructive azoospermia (NOA). Newer advances such as the microdissection-TESE (micro-TESE) technique have continued to build upon past success by improving sperm retrieval and minimizing the postoperative complications compared to TESE. However, even with micro-TESE, sperm retrieval success has ranged from 40% to 60% due to the technique's dependence on surgeon and embryologist experience. While postoperative complications are minimal relative to the traditional TESE technique, testicular tissue must still be extracted without the knowledge of whether sperm are present in biopsies. In this review, we discuss the innovations in the surgical management of men with NOA and describe the novel experimental approaches that can improve sperm retrieval success.

**Key words**: Micro-testicular sperm extraction, nonobstructive azoospermia, testicular sperm extraction, testicular sperm extraction technique

## INTRODUCTION

Nonobstructive azoospermia (NOA) or azoospermia secondary to testicular failure affects approximately 10% of infertile men. The underlying histology of NOA can be sertoli cell-only, maturation arrest, or hypospermatogenesis. Sperm retrieval techniques for men with NOA requires a procedure such as testicular sperm extraction (TESE) or the microdissection-TESE (micro-TESE) followed by intra-cytoplasmic sperm injection (ICSI).[1] Since 1993, TESE was used to successfully retrieve sperm followed by *in vitro f*ertilization (IVF) with ICSI, where only a single sperm is injected into the egg for fertilization.[2] TESE and ICSI provided the first opportunity for men with NOA to father biological children. In 1999, Schlegel showed that sperm retrieval success could be improved from ~40% to ~60% using an operating microscope to identify potential sperm containing seminiferous tubules in a technique known as micro-TESE.[3] While the introduction of the microdissection technique has shown improved success in sperm extraction, there remains much room for improvement in safety, efficacy, and efficiency in sperm retrieval for men with NOA. In this review, we will discuss some of the latest advances on the horizon that have demonstrated the potential to improve sperm extraction success while minimizing the amount of tissue removed and duration of surgery [Table 1].

## TESTICULAR SPERM EXTRACTION/ INTRA-CYTOPLASMIC SPERM INJECTION

TESE for use in IVF with ICSI was first reported in 1993 by Schoysman *et al.*[2] TESE enabled urologists to extract sperm from men who lacked sperm in the ejaculate but had viable sperm in the seminiferous tubules. TESE is performed with a small incision in the tunica albuginea, allowing the

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**Table 1: A comparison of current and experimental surgical techniques and modalities in the management of nonobstructive azoospermia**

| Surgical modalities and techniques | Advantages | Disadvantages |
|---|---|---|
| TESE (current) | Anesthesia not required[3]<br>Can be done in the clinic[3]<br>Good chance of sperm recovery (20-45%)[3] | Large amount of seminiferous tubule removed[4]<br>Increased postoperation inflammation possibly leading to scarring[4]<br>Decreased serum testosterone levels[5]<br>Possible rupture of arterial supply[6]<br>Increased postoperative complications[6] |
| Micro-TESE (current) | Better chance of sperm recovery (45-63%)[4]<br>Less tissue removal[4] | Full operation[3]<br>Anesthesia required[3]<br>More time intensive surgery[3] |
| Multiphoton microscopy (experimental) | Produces 3D *in vivo* histological images[7]<br>Depth of penetration up to 400 um[7]<br>Real-time analysis[7]<br>Minimal damage from low energy laser[8]<br>Proven decreased operating time[8] | Safety of low energy photons unknown[9] |
| Optical coherence tomography (experimental) | Tungsten halogen source safer than laser[10]<br>Produces large 2D histological images[11]<br>Real-time analysis[11] | Absence of cellular and nuclear details[10]<br>Limited depth of imaging[11]<br>Can only image *ex vivo* specimens[10] |
| Raman spectroscopy (experimental) | Flexible probe for *in vivo* surgical ease[12]<br>Nondestructive near infrared source[12] | Diagnostic algorithm not histological image[13]<br>Slow analysis (100 s/spot)[13]<br>Safety of near infrared light unknown[12]<br>Results skewed by light pollution[13,14] |
| Sperm antibody detection (experimental) | Large 2D fluorescent images[14] | Feasibility for ICSI with sperm bound to antibody is not well investigated[14]<br>Safety of injecting antibodies unknown[14]<br>Monoclonal antibodies may not be suitable for a myriad of morphological sperm[14]<br>Can only image *ex vivo* specimens[14] |

TESE=Testicular sperm extraction

surgeon to extract seminiferous tubules. Several tubules are collected and processed for sperm. For men with NOA, TESE provides approximately a 20–45% chance of successful retrieval and can often be done without general anesthesia in the clinic.[3] While convenient and straightforward, TESE removes large sections of the seminiferous tubules and can cause inflammation and potential rupture of the testicular vasculature.[4]

Since TESE collects relatively few sperm, it is nearly always accompanied by assisted reproductive techniques. The most important of these techniques is IVF with ICSI. ICSI, first described in 1992 by Palermo *et al*., is performed when a single sperm is injected into an egg instead of simply placing sperm in a solution with the egg as performed in traditional IVF.[15] The fertilized egg is then placed into the woman's uterus for development. Only a few sperm are necessary for fertilization with ICSI, which enabled techniques such as TESE to be performed for sperm retrieval in the fertility treatment of men with NOA.

## MICRO-TESTICULAR SPERM EXTRACTION

Micro-TESE was first reported in 1999 by Schlegel.[3] Using an operating microscope, the surgeon is able to better visualize regions containing spermatozoa by identifying differences in the size and opacity of seminiferous tubules. Initially, the testes are visualized under 6–8x[3,16] magnification to allow optimal visualization of the blood vessels and a single wide incision in an avascular region of the tunica albuginea is made to expose underlying testicular parenchyma [Figure 1]. Next, under higher magnification (15–25x), the surgeon identifies potential spermatozoa containing tubules and dissects them. Tubules with spermatozoa will appear to be larger, thicker, and more opaque, although this may not be the case in all circumstances since sperm identification is possible only after tubules are biopsied and mechanically processed. The sample is then cut, mechanically processed, and examined by a skilled embryologist for viable spermatozoa. In an initial nonrandomized prospective study, spermatozoa retrieval improved from 45% using TESE to 63% using the microdissection technique.[3] A larger retrospective clinical study with 543 TESE procedures showed similar results with 57% overall spermatozoa retrieval success with the microdissection technique versus 32% with traditional TESE.[4]

While micro-TESE dramatically increases spermatozoa retrieval success, the operation remains a technically challenging one that demands a longer operating time.[17,18] In a retrospective clinical analysis by Ramasamy *et al*. of 793 micro-TESE attempts, mean operative duration ranged from 1.8 to 2.7 h with longer times associated with decreased spermatozoa retrieval success (from 89% to 30% after 2 h).[19] In a retrospective clinical analysis by Ishikawa *et al*., the group showed that in a single urologist

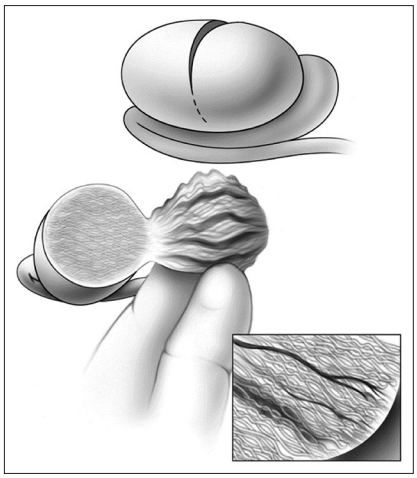

**Figure 1:** Microdissection testicular sperm extraction involves wide incision of tunica albuginea to allow extensive visualization of testicular tubules. Permission for reproduction obtained from Elsevier Publishing[4]

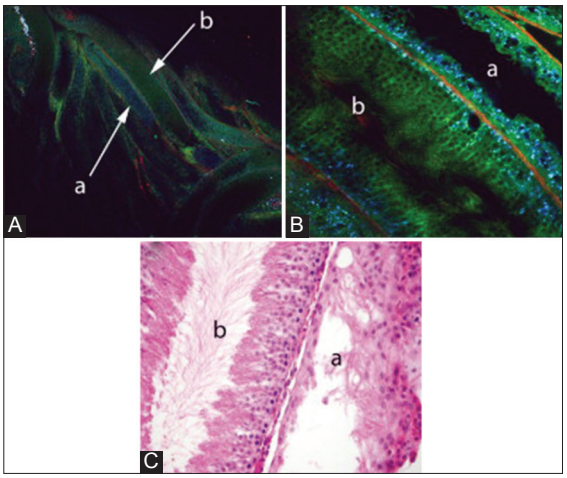


**Figure 2:** Germ cell depleted, sertoli cell-only seminiferous tubule (a) and tubules with spermatogenesis (b). Low-power multiphoton microscopy (A). Reduced from ×4. High-power multiphoton microscopy (B). Reduced from ×25. H and E, reduced from ×25 (C). Permission for reproduction obtained from Elsevier Publishing[7]

performing micro-TESE on 150 men with NOA, sperm retrieval increased from 32% in the first 50 cases to 48% in the last 50 cases with a learning curve of about 50 cases with operating times decreasing from 114 ± 32 min in the first 50 cases to 85 ± 18 min in the last 50 cases.[17] In addition to an experienced microsurgeon, successful spermatozoa retrieval depends on an expert embryologist who is well-versed in processing and analyzing testicular tissues samples for sperm.[7]

In special population, such as men with severe testicular atrophy (testicular volume ≤2 mL) as seen in many patients with Klinefelter's syndrome, smaller testicular volume have not been shown to be a predictor of decreased success with micro-TESE. In a retrospective analysis by Bryson *et al*. 1127 patients with NOA who underwent micro-TESE with ICSI, sperm retrieval rates for patients with testicular volumes of ≤2 mL, 2–10 mL, and >10 mL were 54.7%, 56.25, and 55.1%, $P = 0.53$, respectively.[16] Of the 106 patients with testicular volumes of ≤ 2 mL, 82.2% had Klinefelter syndrome. Though this study encompasses information from a confined population, patients with conditions of severe testicular atrophy such as Klinefelter's should not be discouraged from undergoing the micro-TESE procedure for sperm extraction.

## MULTIPHOTON MICROSCOPY

A challenge faced by micro-TESE use in men with NOA has been the difficulty of successfully differentiating between seminiferous tubules with normal and abnormal spermatogenesis.[8] A technique that can potentially identify the presence of sperm within the tubules without the need for extraction of testicular tissue is multiphoton microscopy (MPM). MPM uses a low energy infrared femtosecond pulse laser with two- and three-photons to produce the excitation of intrinsic molecules causing autofluorescence from intrinsic fluorophores combined with second harmonic generation by supramolecular structures to produce detailed images of underlying tissue.[8,20] The tissue can be optically sectioned allowing for real-time high-resolution images without the conventional use of physical section, labeling, or staining.[8] In one of the earliest studies using MPM to evaluate spermatogenesis in seminiferous tubules, Ramasamy *et al*. showed the ability of MPM to clearly distinguish the underlying microstructure of the seminiferous tubules in a mouse model to allow for sperm detection and histological characterization of individual tubules [Figure 2].[7] In a follow-up pilot study by Najari *et al*., the MPM technique was used in seven men with normal or abnormal spermatogenesis to test the ability of MPM to characterize seminiferous tubules compared to those obtained from hematoxylin and Eosin (H and E) staining.[8] The study showed a 92% concordance rate of diagnosis between the two techniques in men with NOA while showing a concordance rate for all men with normal to be 78%. The study also showed the similar proportions of the categorizations of seminiferous tubules with abnormal versus normal spermatogenesis.

Though the studies on MPM are still in their preliminary phases, the MPM technique has shown promising results that can enhance the ability of the surgeon to improve sperm retrieval by correctly identifying seminiferous tubules with sperm and potentially decreasing the operative time in the process. While it only uses a low energy laser, the MPM laser-guided technique will still need to be evaluated for its long-term safety in future prospective studies as lasers can potentially induce genetic abnormalities in gametes used for IVF with ICSI.

## FULL FIELD OPTICAL COHERENCE TOMOGRAPHY

Full-field optical coherence tomography (FFOCT) is a technique that uses the principle of white light interference microscopy to produce the high-resolution images of unprocessed and unstained excised tissue.[10] In one of the first investigations of the use of commercial prototype use of FFOCT, called Light-CT™, on the quality of histological samples of various rat organs, Jain *et al*. was able to successfully visualize the normal histology of nine different rat organs, including the testes.[10] The images produced by FFOCT allows for the clear recognition of seminiferous tubules, the layers of germ cells that line the tubule, and the distinct tubule lumen with intra-luminal sperm. In one of the first studies of using FFOCT to evaluate spermatogenesis in the seminiferous tubules in a mouse model, Ramasamy *et al*. demonstrated that FFOCT was able to successfully image the seminiferous tubules and determine the status of spermatogenesis.[11] In the pilot animal study, normal rat testes were compared with those that have been injected with two doses of busulfan – an alkylating agent that temporarily arrests spermatogenesis. The FFOCT technique showed seminiferous tubules of relatively uniform size and shape (diameter 328 ± 11 μm) and hair-like structures the indicative of spermatogenesis in normal rat testis samples compared to the heterogeneity in shape and the size of tubules (diameter 178 ± 35 μm) with only 10% of tubules showing normal spermatogenesis in the mice treated with busulfan. The FFOCT findings correlated well with the H and E staining of the same specimens [Figure 3].

The FFOCT technique holds much promise in improving the success of sperm extraction with its potential for reducing the number of biopsies performed and decreasing the operative time for the micro-TESE procedure. The technique allows for rapid imaging of fresh tissue without the need for extrinsic labeling agents. With the halogen lamp as the illuminating power source, the technique offers a relatively safer option compared to laser imaging techniques such as MPM, making it ideal for ICSI as it decreases the chances of photo-damage and mutations in extracted sperm.[11] In addition, the FFOCT technique, compared to traditional confocal microscopy, is much faster (one frame/s) in its generation of images and can cover a relatively larger area of tissue. The major limitations for this technique are the lack of ability to image cellular and nuclear details, its limited depth of imaging, and the constraint of the prototypes to image *ex vivo* specimens only.[10,11] Despite the positive findings in the studies mentioned, FFOCT will still need to be tested in human tissue and further evaluated on its safety and efficacy.

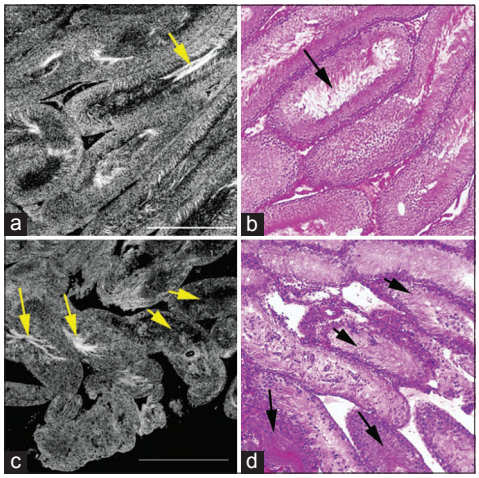


**Figure 3:** Comparative full field optical coherence tomography and H and E-stained histology (a) Testis of a normal rat shows seminiferous tubules with relatively uniform size and shape (b) H and E histology stain of the same specimen. Arrows point to the sperm within the tubule lumen. (c) Seminiferous tubules in the testis of a rat treated with busulfan, showing thinner tubules and a greater degree of heterogeneity in size and shape with ~10% normal spermatogenesis. (d) H and E staining of the same specimen. Field of view in each panel: 1 mm² Permission granted under the creative commons attribution license[11]

## RAMAN SPECTROSCOPY

Raman spectroscopy (RS) is a nondestructive, label-free technique that utilizes laser-based optics to determine the biochemical structures of living tissue. RS uses a low-power, monochromatic laser to excite molecules. Each molecule will in turn uniquely scatter these photons, creating a spectrum specific to each molecular structure. With this technique, investigators can differentiate tissues to generate a "fingerprint" spectrum for each type of tissue. RS is used clinically to identify tumors, analyze serum for oral cancer makers, and detect DNA damage in human sperm.[21-23] In a 2014 study, Osterberg *et al*. demonstrated that this technique can be used to differentiate between tubules with sertoli cell-only histology and tubules with spermatogenesis [Figure 4]. This technique was 96% sensitive and 100% specific in distinguishing the presence of spermatogenesis in the seminiferous tubules of rat models.[13] In another study reported in 2014, Liu *et al*. demonstrated the ability of RS to distinguish between NOA

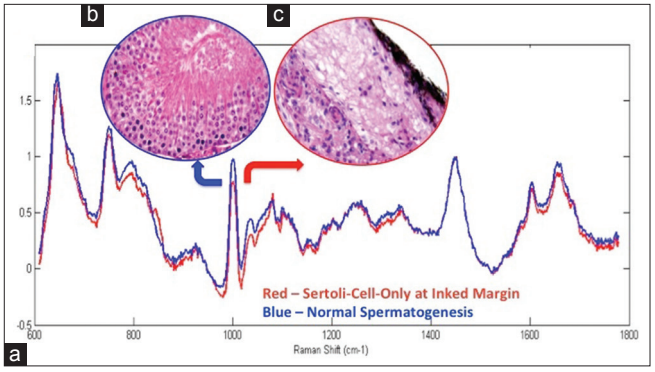


**Figure 4:** (a) Mean processed spectra for sertoli cell-only (red curve) and active spermatogenesis (blue curve) with 1000 and 1690 $cm^{-1}$ discriminatory Raman peak intensity, respectively. (b) Representative testicular biopsy shows active spermatogenesis. (c) Representative testicular biopsy shows sertoli cell-only. (b and c), H and E, reduced from ×200. Permission for reproduction obtained from Elsevier Publishing[13]

and obstructive azoospermia with 90% sensitivity and 85.71% specificity.[12] However, this study was limited in that testicular tissues of men with normal spermatogenesis were not included as controls.

The specificity and sensitivity of RS are greater than any other technique discussed in this review. Further, this technique is nondestructive and the sperm remains viable for ICSI. Conversely, while this technique is touted as nondestructive, the overall safety of RS use on humans has not been thoroughly investigated.[12] Unfortunately, RS is not an imaging modality; instead, the surgeon uses a flexible probe to analyze testicular tissue using an algorithm to decide whether or not to extract tubules. Each analysis currently takes approximately 100 s (~2 min) – thus, while this is a viable real-time analytical tool, it can be inefficient compared to some of the other techniques.[13] Finally, since RS is a light-based spectroscopy, careful fine tuning is required, and excess background lighting can cause errant results, diminishing the reliability of this technique.[13]

## SPERM ANTIBODY DETECTION

The final technique reviewed utilizes fluorescent microscopy to identify antibody-labeled sperm in seminiferous tubules during micro-TESE procedures. Greenhalgh *et al*. first described this technique in 2009 using monoclonal antibodies and a traditional fluorescent microscope.[24] Smith *et al*. improved on this concept in 2012 using a fiber optic confocal fluorescent microscope which has a flexible probe and can be used during surgery to produce *in vivo* cellular images.[14] This technique requires an injection of sperm-specific monoclonal antibodies into the rete testis prior to surgery. Once the testicle has been opened during the micro-TESE procedure, an image of the tubules is produced in < 1 s as the fluorescently labeled sperm are mapped out in the image – thus allowing the surgeon to quickly identify and collect the sperm-filled tubules.

Both studies were based on a mouse-model and have not been validated in human specimens. While the generation of an *in vivo* cellular image would decrease surgical time as well as decrease testicular damage, it remains unclear whether the antibody-bound sperm is viable for assisted reproductive techniques. Further, it is uncertain what the long-term effects are of fluorescent-tagged monoclonal antibodies in humans. Finally, while the monoclonal antibodies bound well to mouse sperm in both studies, it is unlikely that monoclonal antibodies would be suitable for the myriad of morphological sperm found in the human population, thus limiting the effectiveness of this technique.

## CONCLUSION

There is a need for improved ability to identify sperm containing regions within the testis of men with NOA. Currently, skilled surgeons using a microscope during TESE have shown improved sperm retrieval success compared to conventional TESE. Nevertheless, challenges such as long operative duration and the necessity to remove tissue to positively identify sperm limits micro-TESE's wide application. Therefore, it is imperative that we develop a technique using innovative modalities that can achieve similar success but at the same time overcome some of the current challenges. While the safety of the techniques discussed within this review has yet to be fully investigated, we expect future studies to benefit the surgical treatment of men with NOA.

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### *Conflicts of interest*
There are no conflicts of interest.

## REFERENCES

1. Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. Basic Clin Androl 2013;23:5.
2. Schoysman R, Vanderzwalmen P, Nijs M, Segal L, Segal-Bertin G, Geerts L, *et al*. Pregnancy after fertilisation with human testicular spermatozoa. Lancet 1993;342:1237.
3. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999;14:131-5.
4. Ramasamy R, Yagan N, Schlegel PN. Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. Urology 2005;65:1190-4.
5. Andersson AM, Jørgensen N, Frydelund-Larsen L, Rajpert-De Meyts E, Skakkebaek NE. Impaired Leydig cell function in infertile men: A study of 357 idiopathic infertile men and 318 proven fertile controls. J Clin Endocrinol Metab 2004;89:3161-7.
6. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod 1997;12:1688-92.
7. Ramasamy R, Sterling J, Fisher ES, Li PS, Jain M, Robinson BD, *et al*. Identification of spermatogenesis with multiphoton microscopy: An evaluation in a rodent model. J Urol 2011;186:2487-92.
8. Najari BB, Ramasamy R, Sterling J, Aggarwal A, Sheth S, Li PS, *et al*. Pilot study of the correlation of multiphoton tomography of *ex vivo* human testis with histology. J Urol 2012;188:538-43.
9. Rivera DR, Brown CM, Ouzounov DG, Pavlova I, Kobat D, Webb WW, *et al*. Compact and flexible raster scanning multiphoton endoscope capable of imaging unstained tissue. Proc Natl Acad Sci U S A 2011;108:17598-603.
10. Jain M, Shukla N, Manzoor M, Nadolny S, Mukherjee S. Modified full-field optical coherence tomography: A novel tool for rapid histology of tissues. J Pathol Inform 2011;2:28.
11. Ramasamy R, Sterling J, Manzoor M, Salamoon B, Jain M, Fisher E, *et al*. Full field optical coherence tomography can identify spermatogenesis in a rodent sertoli-cell only model. J Pathol Inform 2012;3:4.
12. Liu Y, Zhu Y, Di L, Osterberg EC, Liu F, He L, *et al*. Raman spectroscopy as an *ex vivo* noninvasive approach to distinguish complete and incomplete spermatogenesis within human seminiferous tubules. Fertil Steril 2014;102:54-60.e2.
13. Osterberg EC, Laudano MA, Ramasamy R, Sterling J, Robinson BD, Goldstein M, *et al*. Identification of spermatogenesis in a rat sertoli-cell only model using Raman spectroscopy: A feasibility study. J Urol 2014;192:607-12.

14. Smith RP, Lowe GJ, Kavoussi PK, Steers WD, Costabile RA, Herr JC, *et al*. Confocal fluorescence microscopy in a murine model of microdissection testicular sperm extraction to improve sperm retrieval. J Urol 2012;187:1918-23.
15. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17-8.
16. Bryson CF, Ramasamy R, Sheehan M, Palermo GD, Rosenwaks Z, Schlegel PN. Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. J Urol 2014;191:175-8.
17. Ishikawa T, Nose R, Yamaguchi K, Chiba K, Fujisawa M. Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. Fertil Steril 2010;94:1008-11.
18. Ramasamy R, Reifsnyder JE, Bryson C, Zaninovic N, Liotta D, Cook CA, *et al*. Role of tissue digestion and extensive sperm search after microdissection testicular sperm extraction. Fertil Steril 2011;96:299-302.
19. Ramasamy R, Fisher ES, Ricci JA, Leung RA, Schlegel PN. Duration of microdissection testicular sperm extraction procedures: Relationship to sperm retrieval success. J Urol 2011;185:1394-7.
20. Zipfel WR, Williams RM, Christie R, Nikitin AY, Hyman BT, Webb WW. Live tissue intrinsic emission microscopy using multiphoton-excited native fluorescence and second harmonic generation. Proc Natl Acad Sci U S A 2003;100:7075-80.
21. de Jong BW, Schut TC, Maquelin K, van der Kwast T, Bangma CH, Kok DJ, *et al*. Discrimination between nontumor bladder tissue and tumor by Raman spectroscopy. Anal Chem 2006;78:7761-9.
22. Meister K, Schmidt DA, Bründermann E, Havenith M. Confocal Raman microspectroscopy as an analytical tool to assess the mitochondrial status in human spermatozoa. Analyst 2010;135:1370-4.
23. Sahu A, Sawant S, Mamgain H, Krishna CM. Raman spectroscopy of serum: An exploratory study for detection of oral cancers. Analyst 2013;138:4161-74.
24. Greenhalgh JR, Griffith TS, Wald M. The use of immunofluorescence in microdissection testicular sperm extraction. J Androl 2009;30:548-51.