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RESEARCH HIGHLIGHT

Newly developed techniques in andrology: endoscopy of the vas deference and a new imaging technique for *in situ* localization of vital spermatozoa

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During the past decade, endourology represents the most advanced technology in urology which has provided minimal invasive diagnostic and therapeutic tool for patients with urology diseases. However, because of the much smaller lumen of genital tract, endoscopic andrology remained a dream for andrologists until the first clinical application of vesiculoscopy in laser lithotripsy of seminal vesicle stones in 2006. Lately, Dr Trottmann at Ludwig-Maximilians University, Germany, for the first time established vasoscopy in the seminal duct using a new prototype of a microendoscope and also applied a new imaging technique for *in situ* localization of vital spermatozoa. These newly developed techniques will greatly speed up the clinical practice of endoandrology.

With the development of modern science and technology, there are emerging and promising diagnostic and therapeutic tools for male fertility problems. Transurethral seminal vesiculoscopy is one of the advanced techniques, which has been proved safe and effective in the diagnosis and treatment of ejaculatory duct obstruction, seminal vesicle stones and refractory hemospermia.[1] However, to date, the endoscopy of the vas deference has not yet been achieved because of the small lumen of the seminal duct. As for nonobstructive azoospermia and surgically failed obstructive azoospermia, testicular sperm extraction (TESE) or microsurgical TESE is still the main option for assistant reproduction. The blindly extracted sperms still need to verify their viability *ex vivo* for the use in intracytoplasmic sperm injection. Up to now, there is no imaging technique in the testis which allows the detection of vital spermatozoa, therefore, to improve retrieval rate for the patients undergoing TESE.

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Based on the demanding of above clinical needs in andrology, Trottmann *et al.*[2] at Ludwig-Maximilians University, Munich, Germany established vasoscopy in the seminal duct using a new prototype of a microendoscope. For the first time, they reported a preclinical randomized study at the Twenty-Eighth Annual EAU Congress in Milan on 15–19 March 2013. The vas dereferences of transsexual men were investigated *ex vivo* after surgical removal. In a second step, the vas dereferences of men within 24–48 h after death were investigated *in situ*. For the experiments, a semirigid microendoscope of 0.6 mm outer diameter offering the possibility to insert 0.4 mm thick tools and with integrated fiberoptics (0.9 mm, 10 000 pixels) and a depth of field from 3 to 20 mm was used. After surgical removal, antegrade and retrograde views of the inner lumen of the vas deference were achieved. Using a working channel, a biopsy forceps and a laser fiber were introduced into the inner lumen allowing obtaining probe material and to close or open the lumen by coagulation and vaporization, respectively. The *in situ* investigations showed that transurethral endoscopy of the vas deference provided good images of the inner surface of the seminal duct up to the vesicular gland.

As an emerging optical imaging technology, probe-based confocal laser endomicroscopy (pCLE) enables real-time *in vivo* microscopy of mucosal surfaces during standard endoscopy. With applications currently in the respiratory and gastrointestinal tracts, pCLE has also been explored in the urinary tract for bladder cancer diagnosis.[3–5] Lately, Trottmann *et al.*[6] for the first time evaluated the use of pCLE as a diagnostic tool in andrology.[6] Using pCLE, they set out to optimize sperm retrieval rates in patients undergoing TESE for assisted reproduction by localization of vital spermatozoa in the testis. In a preclinical randomized study, the testes of nine transsexual men, as well of the ejaculates of nine fertile and subfertile men, were investigated by pCLE using the Cellvizio confocal microprobe ProFlexTM S1500 (diameter: 1.5 mm, lateral resolution 3.3 mm) for the investigation of the testes and the microprobe Ultra Mini O (diameter: 2.6 mm, lateral resolution: 1.4 mm) for the examination of the ejaculates (Mauna Kea Technologies, France). Fluorescent labeling was achieved by incubation in (i) 0.01% fluorescein isothiocynate, (ii) 5% cresyl violet solution and (iii) 0.04% acriflavine. The images and movies obtained by pCLE were correlated to the results of confocal laser microscopy, light microscopy and phase contrast microscopy. Fluorescein isothiocynate distinctly marked spermatozoa, spermatocytes and spermatogonia in the seminiferous tubules of the testes. Cresyl violet solution specifically labeled the intercellular matrix in the tubules, whereas acriflavine predominantly stained the nuclei of all cells during spermatogenesis. In the ejaculates, motile spermatozoa were labeled and clearly identified.

Vasoscopy and pCLE might be promising diagnostic and therapeutic tools in andrology. First of all, vasoscopy could be used as a valuable tool for successful treatment of obstructive azoospermia by laser incision or by dilatation of the stricture. Vasoscopy could also be performed for sperm recovery in patients with ejaculatory dysfunction, for example, in patients with injuries of the spinal cord. It makes it possible to obtain probe

material from the seminal duct for histological and microbiological investigations or to locally apply drugs. Moreover, vas deferens embolism also might be inserted and easily removed via vasoscopy instead of current clinical practices of vasectomy and microsurgical vas reversal. So far, pCLE has been used for *in situ* diagnosis of alterations in gastroenterology, pulmonology, ophthalmology and even urology. Trottmann *et al.*[6] for the first time proved this technique may be used as a valuable tool in andrology for the determination of areas in the testis with effective spermatogenesis, thus enabling to optimize the positive sperm retrieval rates in TESE patients. However, the clinical effectiveness of these two technologies is not proven. Further studies are warranted to evaluate the role of these techniques.

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