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REVIEW ARTICLE

Progressing management of non-obstructive azoospermia in the era of microdissection testicular sperm extraction

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Abstract Previously, it was absolutely impossible for azoospermic men to reproduce except in some obstructive azoospermic cases for whom reconstruction of the seminal pathway was successful. However, nowadays, intracytoplasmic sperm injection and microdissection testicular sperm extraction have brought about chances of biological paternity in some non-obstructive azoospermic men. It is almost 15 years since the first trials of testicular sperm retrieval using surgical microscopy for non-obstructive azoospermia were reported. In this manuscript, the progress and outcomes of these novel techniques since then are reviewed, the controversial points are discussed and the latest research to achieve pregnancies in tough non-obstructive azoospermic couples are introduced. Not only the bright side of the renovations, but the underlying concerns are also discussed.

Keywords Azoospermia · ICSI · Microsurgery · Sperm · TESE

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Introduction

The first description of intracytoplasmic sperm injection (ICSI) with testicular sperm was published by Silber in 1994 [1]. Less than a year following this report, high fertilization and pregnancy rates after ICSI with testicular sperm obtained from a testicular biopsy was reported [2]. In this report, testicular sperm was retrieved from men with congenital absence of the vas deferens (CAVD) or irreparable obstructive azoospermia (OA). The first documentation of ICSI with testicular sperm obtained from non-obstructive azoospermia (NOA) was achieved by Devroey in 1995 [3] and again by Silber in 1996 [4]. One of the most common chromosomal abnormalities in azoospermic men is Klinefelter syndrome (KS). KS had been thought to result in absolute sterility, with the exception of some mosaic cases of KS with severe oligozoospermia. However, with testicular sperm extraction (TESE)-ICSI, it is now thought to be one of the most treatable types of sterility.

The first documentation of pregnancies with testicular sperm for nonmosaic Klinefelter syndrome was achieved by Tournaye in 1996 [5]. Consequently, the concept of testicular biopsy has changed from a diagnostic tool to a therapeutic tool. However, the sperm retrieval rate in NOA was unsatisfactory, especially in difficult cases such as Sertoli cell-only (SCO) syndrome, because sampling of testicular tissue is not specific for picking up localized spermatogenesis in these cases. In 1999, Schlegel introduced surgical microscopy to extract more promising seminiferous tubules specifically with testis under direct magnified inspection; this procedure was called microdissection testicular sperm extraction (microdissection TESE) [6]. With this technique, the sperm retrieval rate (SRR) in NOA was improved to 63 % from 45 % with conventional TESE [6]. Thus, a new millennium began with the

innovation of the management of severe male infertility with these novel techniques. In Japan, the first reports of microdissection TESE were published by Okada [7] and Tsujimura [8] in 2002. However, there aren't enough publications in Japan regarding outcomes of microdissection TESE after that. In this review, clinical points and problems of microdissection TESE are overviewed with the introduction of domestic and foreign references reported so far.

Indications for TESE

TESE should be considered in all the patients with azoospermia. In obstructive azoospermia, TESE-ICSI is one of the most promising procedures; another option for the condition is the reconstruction of seminal pathways [9]. In cases of presumed OA, diagnostic testicular biopsy should be performed simultaneously with cryopreservation of testicular sperm. Cryopreservation of testicular sperm may be used in cases in which reconstruction of seminal pathways is cancelled or has failed. In cases of presumed NOA, diagnostic testicular biopsy should not be performed. If the azoospermic patient demonstrates atrophic testis, elevated gonadotropin, or abnormal chromosomes, microdissection TESE should be considered instead of diagnostic testicular biopsy. It has been reported that prior conventional TESE may decrease the chance of retrieval of testicular sperm by following with salvage microdissection TESE, especially if aggressive biopsies were performed in SCO [10].

In cases of occasional spermatozoa, transient azoospermia, cryptozoospermia, and severe oligozoospermia, microdissection TESE may be indicated if ICSI is not possible or is unsuccessful with ejaculated sperm [11]. The presence of ejaculated sperm signifies the existence of limited spermatogenesis in the testis, which can be successfully identified by microdissection TESE. In failed cases of initial ejaculated sperm-ICSI in severely oligozoospermic men and some severely asthenozoospermic men, including immotile cilia syndrome, testicular sperm may improve ICSI outcomes [11]. The advantages of testicular sperm may be derived from their higher DNA integrity level because the DNA fragmentation rate is reported to be lower in testicular sperm compared to ejaculated sperm [12]. However, the quality of retrieved testicular sperm does not always guarantee successful ICSI. In addition, ejaculated sperm sometimes disappear transiently or permanently after TESE. With repeated semen analyses, it is recommended to challenge the cryopreservation of ejaculated sperm as much as possible before introducing microdissection TESE.

In cases of spinal cord injury (SCI), ejaculated sperm can be obtained with electrostimulation or penile vibration [13]. However, the quality of ejaculated sperm is not always satisfactory for intrauterine insemination or in vitro fertilization [14]. Cytokines secreted by leukocytes are responsible for a high DNA fragmentation rate of sperm [15]. TESE-ICSI may be an alternative choice for the SCI couples desiring pregnancies and microdissection TESE-ICSI may be useful in the cases of severely impaired spermatogenesis [16]. Severely impaired spermatogenesis is common in spinal cord injured men regardless of the duration or level of injury [16].

Contraindications for TESE have not been proposed to date; however, in azoospermic men with Y chromosome microdeletions, complete deletion of AZFa, AZFb, AZFa + b, AZFb + c, AZFa + b + c indicate that it is not possible to retrieve testicular sperm with microdissection TESE [17]. In men with AZFc deletions, it has been demonstrated that the deletions could be passed on to male offspring when TESE-ICSI is employed [18]. The future fertility status of these children has not been evaluated; however, a negative impact has not been reported regarding fertilization rate, embryo development, and live birth rate [19]. Recently, short-stature-homeobox (SHOX) aberrations in pseudoautosomal regions (PARs) were reported in infertile men with Y chromosome microdeletions [20]. Genetic testing should be offered to couples desiring pregnancies before performing microdissection TESE and genetic counselling should be offered to the couples on demand [21, 22]. If selection is allowed in embryos depending on gender, it is possible to prevent transmission of AZF deletion [23]. While the chance of impregnation in a non-mosaic 47, XXY karyotype has been improved with microdissection TESE-ICSI, no cases of successful testicular sperm retrieval from 46, XX males have been reported.

In azoospermic cases with a history of orchiopexy, microdissection TESE-ICSI may be an appropriate choice [24, 25]. It may not be prudent to perform TESE in cases of undescended testis, because a few single case reports demonstrated that ejaculated sperm appeared after orchiopexy in matured azoospermic men [26, 27]. Still, it has not been reported that testicular sperm was successfully retrieved from inguinal or abdominal testis. In undescended testis, orchiopexy may have to be performed initially and followed by microdissection TESE. However, there is no evidence that orchiopexy after puberty recovers spermatogenesis [28].

If a varicocele exists in NOA, varicocelectomy should be initially considered [29]. Despite the controversy regarding performing a varicocelectomy in cases of NOA [30, 31], after varicocelectomy, ejaculated sperm has been reported to be present in 39 %; furthermore, 15 % of these cases achieved natural conception [32]. Even in cases of persistent azoospermia, the testicular sperm retrieval rate may be improved after varicocelectomy. However, the

effects may be limited in cases of SCO and early maturation arrest [33]. In these cases, a prior simple testicular biopsy may be useful to determine the indication for a varicocelectomy in NOA.

In cases of idiopathic hypogonadotropic hypogonadism, TESE should not be performed; administration of human chorionic gonadotropin (hCG), human menopausal gonadotropin (hMG) or recombinant follicle stimulating hormone (rFSH) should be considered [34]. Without gonadotropin administration, TESE may fail; supplementation with gonadotropins usually restores spermatogenesis and sometimes enables natural conception [35].

Complications of TESE

TESE is a comparatively safe procedure. It can be performed under local anesthesia. However, microdissection TESE sometimes requires a longer surgery time and wider dissection of the tunica albuginea [36]. In cases of NOA in which microdissection TESE is considered, epidural anesthesia or general anesthesia should be considered if they are accessible. Postoperative pain is a common complications; however, it is reported that use of perioperative celecoxib decreases postoperative pain and opioid use. [37].

Testicular atrophy may be one of the main complications of TESE; therefore, it is necessary to preserve the arteries under the tunica albuginea as much as possible. Microdissection TESE has the advantages of sparing arteries and controlling bleeding under direct inspection. A lower complication rate has been reported with microdissection TESE rather than simple/conventional TESE [38].

Serum hormonal levels after microdissection TESE have not been well documented [38]. Testosterone replacement will be necessary in some cases after microdissection TESE; therefore, hormonal surveillance is recommended after TESE, especially in cases of small testis or solitary testis [39]. In most Klinefelter syndrome cases, testosterone replacement will be necessary after TESE [40, 41].

To date, the apparent risk of congenital anomalies or developmental disorders has not been reported in the offspring of NOA couples who underwent TESE-ICSI. However, inheritance of AZF deletions or unidentified autosomal genetic disorders may cause sterility in the second generation. There are many genes on the autosomes and the X chromosome related to spermatogenesis [42]. This implies that TESE-ICSI may produce carriers of male infertility regardless of gender. In Klinefelter syndrome cases, the chromosomal phenotype of male offspring produced via TESE-ICSI has been reported to be near-normal [43]. However, the concern exists that chromosomal quantity disorders might appear in the next generation of infants produced from Klinefelter syndrome cases.

Microdissection TESE vs. conventional/simple TESE and multiple fine needle biopsy

Microdissection TESE has an advantage in sperm retrieval rate, surgical control, and pregnancy rate compared with other procedures such as simple/conventional TESE and multiple needle biopsies [44, 45]. Even in primary conventional TESE failure, some cases can be rescued by salvage microdissection TESE [46]. In obvious NOA cases which demonstrate either atrophic testis, elevated FSH, or Klinefelter's (47, XXY), diagnostic testicular biopsy should not be performed. Microdissection TESE should be considered prior to conventional/simple TESE in these cases. Microdissection TESE requires a surgical microscope, an experienced embryologist and skilled surgeons. The learning curve for microdissection TESE was reported by Ishikawa [47]. The sperm retrieval rate plateaued at 100 cases according to this single surgeon's experience [47].

Turek reported an excellent sperm retrieval rate with systematic fine needle testicular aspirations [48]. This procedure is quite convenient and does not require skills or expensive equipment. However, the risk of vascular injury cannot be completely avoided. Systematic fine needle aspirations may fail to pick up testicular sperm from testis with limited production. Because spermatogenesis is usually focal rather than diffuse in NOA cases represented by SCO [49], systematic fine needle testicular aspirations will have less of an advantage under these circumstances. However, there might be racial differences in the pathogenesis of non-obstructive azoospermia, which should be elucidated in the future.

In performing microdissection TESE, one of practical concerns is which side or both sides testis should be explored. It is reported that only 8 % of men who underwent bilateral testicular microdissection had sperm found on the contralateral side when no sperm were identified on the initial side. On the other hand, in men with Klinefelter syndrome the chance of sperm retrieval was higher on the contralateral side after negative unilateral microdissection [50].

In conclusion, if conditions are favorable, microdissection TESE may be the gold standard for most NOA cases.

Timing of testicular sperm retrieval

It is speculated that most doctors perform TESE followed by controlled oocyte stimulation/oocyte retrieval because there is no guaranteed predictor for the retrieval of testicular sperm [51]. Normal sized testis and normal gonadotropin levels do not necessarily guarantee the success of testicular sperm retrieval; furthermore, high serum FSH levels and atrophic testis do not eliminate the possibility of testicular sperm retrieval.

In OA, there may be a few disadvantages to freezing and thawing testicular sperm [52]. However, in NOA, there may be some disadvantages in freezing and thawing testicular sperm due to its poorer quality and quantity [53]. If fresh testicular sperm is used for ICSI in NOA couples, the fertilization rate and clinical pregnancy rate are similar to those in OA couples [49]. In scheduling concomitant TESE, the oocyte retrieval date cannot be decided in advance; thus, it requires both the male partners and surgeons to stand by until oocyte retrieval is performed. Even with controlled ovarian stimulation, it is impossible to predict the precise oocyte retrieval date in advance because the maturation of the follicles varies.

In order to overcome the difficulties in scheduling concomitant TESE-ICSI, vitrified/warmed oocytes-TESE-ICSI has been attempted. It has been reported that the outcomes of TESE-ICSI using vitrified/warmed oocytes and fresh testicular sperm were comparable to those using fresh oocytes and fresh testicular sperm [54]. However, if testicular sperm retrieval fails, fresh oocytes or vitrified oocytes are wasted. To avoid wasting oocytes, some investigators have reported the fertilization of these oocytes with donor sperm [55, 56]. However, this course of action should be performed under ethical considerations.

Recently, the sperm vitrification technique has been reported [57]. With a successful vitrification technique using a small number of vulnerable testicular sperm, TESE can be performed prior to oocyte retrieval in all cases. In addition, to avoid unnecessary ovarian stimulation and oocyte retrieval, reliable predictors of testicular sperm retrieval in NOA should be developed [58].

Pretreatment prior to TESE

As previously mentioned, TESE should not be performed in cases of hypogonadotropic hypogonadism. Supplementation of gonadotropin should first be attempted for at least 6 months in these cases. Conversely, administration of gonadotropin in normogonadotropic or hypergonadotropic hypogonadism may be less effective. However, recent trials suggest that this pretreatment may be beneficial in some limited cases such as hypospermatogenesis [59]. However, in most hypospermatogenesis instances, testicular sperm retrieval was successful without pretreatment by microdissection TESE [49]. In Klinefelter syndrome, it has been reported that administration of hCG, aromatase inhibitor, or clomiphene citrate benefitted patients with a low testosterone level [60]. In cases of severely impaired spermatogenesis such as SCO or early maturation arrest, there is no evidence that demonstrates a benefit of any pretreatment prior to microdissection TESE. In patients with undescended testis, orchiopexy should be initially performed [25]. At least 6 months after orchiopexy, microdissection TESE should be considered if persistent azoospermia is observed [24]. In patients with a varicocele, varicocelectomy may be considered first [29, 32, 33]. However, it has been reported that limited cases such as hypospermatogenesis or late maturation arrest will benefit from a varicocelectomy [31]. This implies that in NOA with varicocele, a diagnostic biopsy may be useful to predict the effect of varicocelectomy prior to microdissection TESE. If the patient demonstrates hyperprolactinemia with low FSH, it is necessary to diagnose the underlying endocrine disease [61, 62]. Oral bromocriptine should be initially attempted; however, surgical treatment will be required if it is ineffective [61, 62].

Prediction of testicular sperm retrieval

To date, there is no specific marker that predicts the success of testicular sperm retrieval or a diagnosis of OA/NOA [63]. The FSH level and testicular volume can estimate the likelihood of testicular sperm retrieval; however, they cannot always predict success or failure prior to TESE [63]. It is demonstrated that patient age, Klinefelter syndrome and history of cryptorchidism were significant predictors of sperm retrieval [64]. The most reliable marker may be diagnostic pathology measured by Johnsen's score [65, 66]. However, this information is obtained via a testicular biopsy, which is considered to be contraindicated in obvious NOA. Chromosome analysis and genetic tests such as Y chromosome microdeletion may be useful to exclude some cases in which microdissection TESE will fail [17].

Future aspects

Novel technologies such as ICSI and development of microdissection TESE have provided many opportunities for biologic fatherhood in many azoospermic couples in whom success was deemed to be absolutely impossible. However, many severe cases cannot be rescued even with these treatments. In maturation arrest cases, some cases may respond to previously described pretreatment. However, development of an in vitro maturation system is under development [67]. In SCO cases in which microdissection TESE is not successful, it is necessary to produce germ cells from somatic cells [68].

These revolutionary techniques of handling and managing cells in vitro may prove to be a breakthrough for currently untreatable couples. However, the mechanism regarding how impairment of spermatogenesis or absence of germ cells in testis occurs, as well as the mechanism why heterogeneity of spermatogenesis in testis sometimes occurs, should be

elucidated. The reason why heterogeneity of spermatogenesis in testis occurs could be partially explained by genetic or chromosomal mosaicism [69]. If this is the case, microdissection TESE may not transmit a male sterile phenotype to the next generation. Another possible presumed explanation would be the failure of migration and equal distribution of gonocytes in primitive testis, which might develop localized spermatogenesis in SCO [70].

However, if a male sterility factor is transmitted to offspring with TESE-ICSI, this procedure may result in sterile carriers [22]. This implies that more couples will require artificial techniques to reproduce. So far, with Y chromosome microdeletions, not only future impaired fertility but increased risk of medical problems and congenital abnormalities are suggested [20]. In order to insure that TESE does not spread sterility factors to subsequent generations and does not conflict with natural selection via sterility, tracing and surveillance of TESE-ICSI children may be encouraged separately from total modern ART outcomes.

Conflict of interest Satoru Kanto, Kazumitsu Yamasaki and Teruaki Iwamoto declare that they have no conflict of interest.

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