Dear Editor,

Microdissection testicular sperm extraction (micro-TESE) is an optimal sperm extraction method for men with nonobstructive azoospermia (NOA).1 According to a recent systematic review by Corona et al.,2 the sperm retrieval rate by micro-TESE and live birth rate per intracytoplasmic sperm injection (ICSI) cycle among NOA patients were 46% and 24%, respectively. In cases where sperm can be collected from NOA patients, the live birth rate remains low. If the potential outcomes of ICSI can be predicted by sperm motility assessment, such as pregnancy not anticipated because of immotile sperm, unnecessary ovarian stimulation or oocyte retrieval from female partners could be avoided.

Several previous studies have reported contrasting outcomes from TESE-ICSI in relation to sperm motility.3–6 Some have demonstrated that sperm motility has no effect on ICSI outcomes,3 whereas others have shown that motile spermatozoa are superior to immotile spermatozoa.3,4 In these studies, cases with female infertility factors were not excluded. In some studies, the ICSI3 or embryo transfer (ET)6 cycles were compared instead of sperm-injected oocytes; however, this may lead to an inaccurate assessment of the sperm motility effect on ICSI outcomes.

Therefore, in this study, we excluded subjects with female infertility factors and compared the fertilization, good embryo, and pregnancy rates for oocytes injected with motile or poorly motile sperm. This retrospective study reports the effect of the motility of sperm extracted by micro-TESE on fertilization, good embryo formation, and pregnancy rates per ICSI cycle among NOA patients.

Selected infertile couples had males who were diagnosed with NOA and underwent sperm extraction by micro-TESE followed by ICSI at the Department of Reproductive Medicine of the Yokohama City University Medical Center, Yokohama-shi, Japan, between January 2009 and December 2019. Written informed consent was obtained from all included patients. The study protocol was approved by the Yokohama City University Certified Institutional Review Board (approval number: B210600082). Patients with female partners over 40 years of age or with infertility factors, such as endometriosis and premature ovarian failure, were excluded.

Extracted sperms were cryopreserved regardless of motility and thawed before use in ICSI. We performed 16 oocyte collection cycles and collected 129 metaphase II oocytes. An embryologist assessed sperm motility as forward moving or poorly motile. The poorly motile sperm group included immotile and nonforward moving spermatozoa. Motile sperms were injected into 69 oocytes (one sperm per oocyte), and poorly motile sperms were injected into 60 oocytes (one sperm per oocyte; 27 immotile and 33 nonforward moving). The mechanical touch technique was used to confirm sperm viability in the context of ICSI for immotile sperm.

An oocyte was considered fertilized by microscopic observation of two pronuclei 16–20 h after ICSI. Embryo quality was assessed using the Veeck classification at 2 days or 3 days postoocyte collection and the Gardner and Schoolcraft blastocyst grading system at 5 days postoocyte collection. A single good embryo was transferred into the uterus of each female partner at the 4- to 8-cell or blastocyst stage at 2 days, 3 days, or 5 days postoocyte retrieval. All remaining good embryos were cryopreserved. Pregnancy was defined as the observation of a gestational sac in the uterus by ultrasonography.

Fertilization, good embryo, and pregnancy rates/ET cycles were compared between motile and poorly motile sperm. Statistical analysis was performed using Excel (version 16.0, Social Survey Research Information Co., Ltd., Tokyo, Japan) and JMP (version 13.2.0, SAS Institute, Cary, NC, USA). Student’s t-test, Chi-squared test, and analysis of variance (ANOVA) were used to compare values wherever appropriate. Results were considered significant if P < 0.05.

Patients’ background and reproductive data for couples in each collection cycle are shown in Table 1. The mean age of males was 37 (standard deviation [s.d.]: 6, range: 29–49) years, while that of their female partners was 32 (s.d.: 5, range: 25–39) years.

We observed a significant difference in the fertilization rate between motile sperm-injected (66.7%) and poorly motile sperm-injected (45.0%) oocytes (P = 0.013; Supplementary Figure 1A). No significant
differences were observed in the good embryo and pregnancy rates (Supplementary Figure 1b and 1c).

In contrast to previous studies based on simple motility classification (motile or nonmotile), our study classified sperm based on progressive motility (forward moving or poorly motile). Consistent with the findings of Nagy et al. and Park et al., we report that decreased sperm motility led to a decreased fertilization rate. We speculate that sperms with poor motility have reduced functional capabilities and cannot easily activate the oocyte and induce fertilization.

Spermatozoa are produced in the testes then mature and acquire motility as they pass through the epididymis. Unlike ejaculated sperm or sperm in the epididymis, it is difficult to judge testicular sperm quality by their motility. Other ways to select viable sperm include using pentoxifylline and a hypo-osmotic swelling test. In particular, pentoxifylline is easy to use and has been widely used in recent years, but there are concerns about its toxicity to oocytes or embryos. Navas et al. reported that the use of pentoxifylline to identify viable spermatozoa did not increase adverse effects on obstetric and neonatal outcomes, and it was expected to be a useful sperm selecting method. Although poorly motile sperm induced lower fertilization rates, there were no significant differences in embryonic development and pregnancy rates between oocytes fertilized by motile and poorly motile sperm after fertilization was achieved. This suggests that poorly motile sperm from testes can produce intact embryos via micro-TESE-ICSI. We also speculate that there is a weak correlation between the genetic information of spermatozoa and testicular sperm motility of NOA patients.

One limitation of our study was the small number of subjects. More studies must be designed to accurately assess the impact of sperm motility on the outcome of ICSI for larger numbers of patients.

In our study, oocytes injected with poorly motile sperm showed a low fertilization rate. These data may be presented to NOA couples to help them decide whether or not to perform ovulation induction and oocyte retrieval (invasive procedures). Conversely, our data also suggest that sperm motility does not significantly affect embryo development and pregnancy rates after fertilization. Additionally, TESE-ICSI is the only opportunity for couples with NOA to conceive using their own gametes. Therefore, these two aspects should be presented and carefully discussed with the couple in the decision-making process.

**AUTHOR CONTRIBUTIONS**

YA, MM, HI, KT, TH, and HS designed the study. YA and MM collected the data. YA and MM analyzed the data and wrote the manuscript. EM and YY supervised the study. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

All authors declare no competing interests.

Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.

**REFERENCES**

1. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999; 14: 131–5.
2. Corona G, Minhas S, Giewercman A, Bettocchi C, Dinkelman-Smit M, et al. Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis. Hum Reprod Update 2019; 25: 733–57.
3. Nagy ZP, Joris H, Verheyen G, Tournaye H, Devroey P, et al. Correlation between motility of testicular spermatozoa, testicular histology and the outcome of intracytoplasmic sperm injection. Hum Reprod 1998; 13: 890–5.
4. Konc J, Kanyê K, Cseh S. The effect of condition/state of testicular spermatozoa injected to the outcome of TESE-ICSI-ET cycles. Eur J Obstet Gynecol Reprod Biol 2008; 141: 39–43.
5. Park YS, Lee SH, Song SJ, Jun JH, Koong MK, et al. Influence of motility on the outcome of in vitro fertilization/ intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. Fertil Steril 2003; 80: 526–30.
6. Hessel M, Robben JC, D’Hauwers KW, Braat DD, Ramos L. The influence of sperm motility and cryopreservation on the treatment outcome after intracytoplasmic sperm injection following testicular sperm extraction. Acta Obstet Gynecol Scand 2015; 94: 1313–21.
7. Veecck LL. Preembryo Grading and Degree of Cytoplasmic Fragmentation. An Atlas of Human Gametes and Conceptuses: An Illustrated Reference for Assisted Reproductive Technology. Boca Raton: CRC Press; 1999. p46–51.
8. Navas P, Paffoni A, Intra G, Gonzalez-Uchor A, Clavero A, et al. Obstetric and neonatal outcomes of ICSI cycles using pentoxifylline to identify viable spermatozoa in patients with immotile spermatozoa. Reprod Biomed Online 2017; 34: 414–21.
9. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 1997; 56: 602–7.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

©The Author(s)(2021)
Supplementary Figure 1: (a) Fertilization rates between motile and poorly motile sperms. (b) Good embryo formation rates† between motile and poorly motile sperms. (†Calculated as the number of good embryos/the number of fertilized egg × 100%). When embryo quality was assessed 2 days post-oocyte collection, the good embryo group included those at 4-cell Grades 1–3, 5-cell Grade 1, and 6-cell Grade 1 stages; at 3 days post-oocyte collection, 8-cell Grades 1–3, 9-cell Grades 1–3, and 10-cell Grade 1 stages; at 5 days post-oocyte collection, ≥3BB blastocysts. (c) Pregnancy rates between motile and poorly motile sperms.