New device for sperm preparation involving migration-gravity sedimentation without centrifugation compared with density-gradient centrifugation for normozoospermic intrauterine insemination

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Objective: To investigate the efficacy of a new device for sperm preparation involving migration-gravity sedimentation without centrifugation (MIGLIS), compared with density-gradient centrifugation (DGC) for normozoospermic intrauterine insemination (IUI).

Design: Retrospective cohort study.

Setting: Not applicable.

Patients: A total of 10,318 cases of IUI (3,015 MIGLIS and 7,303 DGC) between October 2013 and September 2019.

Interventions: None.

Main Outcome Measures: Sperm analysis, subsequent pregnancy outcomes, and complications.

Results: MIGLIS was associated with a lower sperm recovery rate and fewer injected sperm compared with DGC. However, the overall pregnancy rates following MIGLIS and DGC were similar (MIGLIS 8.8%, DGC 9.3%). In a subanalysis according to age, the pregnancy rate was higher for MIGLIS among women 40–41 years of age (8.6% vs. 5.9%). Peritonitis was the only recorded complication, with similar frequencies in the MIGLIS and DGC groups (MIGLIS two cases, DGC four cases). No cases became severe, and all improved after antibiotic treatment. There were no cases of uterine cramping or pain symptoms.

Conclusions: MIGLIS is a new sperm preparation method that does not require centrifugation. Its use was associated with pregnancy rates similar to those with DGC and a higher pregnancy rate in older women. MIGLIS is a novel sperm preparation method for selecting spermatozoa with high motility and good fertilization ability in patients undergoing IUI, in vitro fertilization, and intracytoplasmic sperm injection. (Fertil Steril Rep® 2020;1:106–12. ©2020 by American Society for Reproductive Medicine.)

Key Words: MIGLIS, centrifugation, sperm preparation, sperm DNA, intrauterine insemination

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fertilization by enzymes in the oocyte cytoplasm (5), studies of donor oocytes have shown that the repair ability depends on the donor’s age (6). DGC is therefore likely to have a greater negative impact in older women who would like to achieve pregnancy. To date, DGC has been an indispensable method in sperm preparation, but new sperm preparation methods that avoid centrifugation and the associated DNA damage would be advantageous, especially in older women.

The migration gravity sedimentation method (MGS), which uses sperm migration and gravity sedimentation to collect motile sperm without centrifugation, was first reported in 1983 (7). We subsequently improved MGS and reported good results for intrauterine insemination (IUI) in 1988 (8). However, mass production of disposable devices for MGS was not possible at that time, and DGC thus became the mainstream technique for sperm preparation. However, such production has recently become possible. Therefore, we have developed improved disposable tubes for MGS in collaboration with Menicon Co., Ltd., a leading manufacturer of devices using biocompatible materials, and developed a device (MIGLIS) to enable more efficient sperm collection.

To use MIGLIS, a liquefied semen sample is injected into the tube and left to stand for 1 hour at room temperature and normal gas phase. This procedure provides good-quality sperm without the DNA damage caused by centrifugation. Furthermore, the procedure reduces staff time required for sperm preparation, thus allowing IUI to be performed in small clinics. MIGLIS could also be used in specialist infertility clinics to select sperm without the centrifugation-induced DNA damage associated with DGC, potentially resulting in higher sperm motility and greater fertilization ability. MIGLIS may therefore be a valuable sperm preparation tool for IUI, in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). In this study, we compared the validities of MIGLIS and DGC for sperm preparation and investigated the safety, efficacy, and complications of MIGLIS for routine use.

MATERIALS AND METHODS
Study Design
This study was approved by the ethics committee of the Japanese Institution for Standardizing Assisted Reproductive Technology (Approval No. 202002). Statistical analysis was performed after anonymizing patient data. Patients provided written informed consent for sperm preparation by both MIGLIS and DGC.

Study Design and Data Collection
A total of 22,455 IUI procedures were performed at a private Japanese clinic from October 2013 to September 2019. Among these, 73 samples used thawed sperm and 35 nonadjusted samples were excluded. A total of 6,086 samples had motile sperm concentrations (sperm concentration × motility) < 10 × 10^6/mL, which were prepared by the small-scale Percoll two-layer method and were excluded from the cohort. A further 1,161 cases involving women ≥ 44 years of age were excluded because of their low pregnancy rate. We included up to 10 IUI procedures (we reported comparable pregnancy rates per IUI among the first 10 IUI treatments [9]), and 764 cases with ≥ 11 IUI procedures were excluded. A total of 1,180 cases with unknown IUI results and 27 cases using nonstandard induction methods (categorized as others) were excluded. Of the remaining 13,129 cases of IUI, 2811 with a semen volume > 3.0 mL were excluded to ensure similar conditions for MIGLIS and DGC. We finally analyzed 10,318 cases of IUI (3,015 MIGLIS and 7,303 DGC).

Our clinic started using IUI and IVF in 1991, and has been active in the clinical front line of infertility treatment in Japan ever since. DGC was used as a sperm preparation method for IUI until May 2017, and MIGLIS was subsequently introduced in June 2017. We initially used both methods in parallel and compared the results. We assigned each embryologist to perform either MIGLIS or DGC for sperm preparation for IUI, and the sperm preparation method was determined according to the embryologist in charge on the day. After confirming that MIGLIS offered a good alternative to DGC, we increased the ratio of MIGLIS use. Using MIGLIS, if the semen volume was > 3.0 mL, only 3.0 mL was extracted and used for IUI, whereas the semen volume was not adjusted for DGC, leading to potential bias. We therefore omitted cases with a volume > 3.0 mL for both MIGLIS and DGC in this study. However, we conducted a subanalysis of cases including a semen volume > 3.0 mL. We also conducted a subanalysis to adjust for historical influence and matching the total cohorts of MIGLIS and DGC, restricting the target period from March 2016 to September 2019. This clinic administered prophylactic cephalexin after IUI to prevent endometritis.

Sperm Preparation for MIGLIS
The MIGLIS device comprises a small conical cup (central tube) built into an outer container (Fig. 1A). The procedure is shown in Figure 1B. Liquefied semen is injected into the space between the outside of the cup and the inside of the container, near the upper edge of the cup. If the semen volume is insufficient, a spacer is used to allow the semen to reach near the upper edge of the cup. The inner lid is placed and phosphate–buffered saline solution (PBS) is poured gently from the bottom of the cup, overflowing the upper edge of the cup, to cover the semen. The inner lid is placed to prevent the semen from flowing over the upper edge of the ruffled cup to the bottom of the cup when injecting the PBS. The device is then covered and left to stand for 1 hour at room temperature and normal gas conditions (iv). The sperm swims up to the PBS and falls to the bottom of the cup by gravity when it crosses the upper edge of the cup (the mechanism shown in Fig. 1C). After 1 hour, 0.5 mL of the sperm suspension is collected from the bottom of the cup using a tuberculin syringe with a needle. The needle is then replaced with an IUI catheter to perform IUI.

Sperm Preparation for DGC
DGC for sperm preparation was performed as described previously (9). Briefly, semen was overlaid on 4 mL of 90% Percoll, and the interface between the semen and the Percoll was stirred to create a density gradient. After centrifugation at
600 × g for 20 minutes, high-density sperm were precipitated at the bottom. The supernatant was then removed, and 2.5 mL of PBS was added to the precipitated spermatozoa, stirred, and centrifuged at 200 × g for 5 minutes. A further 0.5 mL of PBS was then added to the precipitated spermatozoa and stirred. This sperm suspension was used for IUI using a tuberculin syringe and an IUI catheter.

**Adaptation for IUI and Selection of Ovulation Induction Agent**

Our clinic performs IUI in women with at least one passable fallopian tube. The indications for IUI include male factor, sexual dysfunction, cervical factor, and unexplained infertility. Male factor infertility, defined as a motile sperm concentration <10 × 10⁶/mL, was treated with sperm preparation using the small-scale Percoll two-layer method and was excluded from this cohort. Sexual dysfunction included erectile disorder, ejaculatory disorder, and dyspareunia. Cervical factor was defined as normal semen findings but poor results in postcoital tests. The clinic has adopted the step-up method, and IUI was carried out according to the following criteria: women <35 years of age who fail to become pregnant after six episodes of timed intercourse; and women ≥35 years of age who fail to become pregnant after three episodes of timed intercourse. Unexplained infertility included cases not identified as male factor, sexual dysfunction, or cervical factor, and included cases for which timed intercourse failed to result in pregnancy.

Regarding the choice of ovulation induction method, the ovulation induction method used in our clinic did not aim to achieve superovulation but rather aimed for single-follicle ovulation in patients with ovulation disorders. We previously found comparable pregnancy rates in women undergoing natural cycle–induced and clomiphene citrate–induced IUI (which is generally considered to have a higher pregnancy rate), whereas natural cycle IUI could reduce the multiple pregnancy rate (9). Women without ovulation disorders therefore underwent natural cycle IUI, whereas in women with an ovulation disorder clomiphene citrate was the first-choice induction agent. In the event of side effects from clomiphene citrate such as thinning of the endometrium or failure of five attempts with clomiphene, induction was changed to an aromatase inhibitor. Our clinic used letrozole (2.5-mg tablet; Fuji Pharma Co., Ltd.) as an aromatase inhibitor. Human menopausal gonadotrophin and cyclofenil were used if necessary for ovulation induction.
Statistical Analysis

Pregnancy was defined as confirmation of a gestational sac in the uterus by transvaginal ultrasound. Statistical analysis was carried out using SPSS version 26 (IBM Corp.), graphs were drawn using GraphPad Prism 8 (GraphPad Inc.), and images were adjusted using Adobe Illustrator CC (Adobe Inc.). Fisher’s exact probability test and Mann–Whitney U tests for continuous variables were used. A P value <.05 was considered statistically significant.

RESULTS

A total of 10,318 IUI procedures carried out between October 2013 and September 2019 satisfied the criteria, including 3,015 cases of MIGLIS and 7,303 cases of DGC. Semen volume, sperm concentration, and sperm motility were comparable between the MIGLIS and DGC groups, but the sperm recovery rate (MIGLIS vs. DGC, 13.84% vs. 25.09%, P < .01) and injected sperm count (MIGLIS vs. DGC, 12.7 × 10^6 vs. 24.1 × 10^6, P < .01) were significantly lower in MIGLIS in pregnant cases (Table 1). The ages of the pregnant women were similar in both groups, but the variance was slightly larger in the MIGLIS group (mean ± standard deviation 35.6 ± 3.72 vs. 35.5 ± 3.33) (Table 1). Overall pregnancy rates were comparable between the MIGLIS and DGC groups (8.8% vs. 9.3%, respectively, P = .252) (Table 2). However, MIGLIS was associated with a significantly higher pregnancy rate than DGC in women 40–41 years of age (8.6% vs. 5.9%, respectively, P = .043) (Table 2, Fig. 2). The pregnancy rate decreased markedly with increasing maternal age in both groups, but MIGLIS tended to maintain pregnancy rates in older women better than DGC.

Regarding the use of ovulation induction agents, natural cycle, clomiphene citrate, and aromatase inhibitors did not affect the pregnancy rate using either MIGLIS or DGC in the small sample included in this study. We conducted a subanalysis of women 40–41-years of age stratified by ovulation induction agent to determine the effect of the ovulation induction agent on the pregnancy rate. The pregnancy rate was comparable in patients treated with an ovulation induction agent compared with natural cycles, both overall (6.5% and 6.9%, respectively, P = .438) and in cases treated with MIGLIS or DGC (Supplemental Table 1), indicating that the use of an ovulation induction agent did not increase the pregnancy rate. To avoid multiple pregnancies, our clinic has performed unstimulated IUI for ovulatory women since 2006, and ovulation induction was limited to patients with ovulatory disorders, accounting for the low rates of ovulation induction (22%) and multiple pregnancies (1.5%) throughout the study.

Regarding the infertility diagnosis, MIGLIS resulted in a significantly higher pregnancy rate than DGC in patients with sexual dysfunction (MIGLIS vs. DGC, 15.7% vs. 10.2%, P < .041), a similar rate in patients with unexplained infertility (MIGLIS vs. DGC, 8.6% vs. 9.0%, P = .313), and a significantly lower pregnancy rate in patients with cervical factor infertility (MIGLIS vs. DGC, 6.8% vs. 10.8%, P < .015).

Peritonitis was the only complication, with similar frequencies in the MIGLIS and DGC groups (two cases vs. four cases, respectively, P = .824). No cases were severe, and all improved following antibiotic treatment. Although MIGLIS could not completely remove seminal plasma components, no patients complained of uterine cramping or pain symptoms, suggesting that the content of seminal plasma components may have been very small.

We also validated our results in two subanalyses. We analyzed cases including a semen volume >3.0 mL (Supplemental Tables 2 and 3). The overall pregnancy rate did not change, and MIGLIS tended to result in a higher pregnancy rate in women 40 to 41 years of age. The recovery rate and number of sperm injected were also relatively unchanged. We also conducted a subanalysis restricting the target period from March 2016 to September 2019, to minimize the historical influence and match the MIGLIS and DGC cohorts (Supplemental Tables 4 and 5). The overall pregnancy rate of DGC increased from 9.3% to 9.7%, whereas the pregnancy rates of women 38 to 39 and 40 to 41 years of age with DGC increased and the pregnancy rate for patients <35 years of age decreased. MIGLIS still tended to result in a higher pregnancy rate in women 40 to 41 years of age. Mean age and sperm parameters were comparable between the two periods.

DISCUSSION

The selection of better sperm during sperm preparation is an important factor in fertility treatment. The current results showed that despite a lower recovery rate and injected sperm...
| Characteristic                  | Total cycles | MIGLIS | DGC |
|-------------------------------|--------------|--------|-----|
|                               | pregnant (total) | Pregnancy rate (%) | pregnant (total) | Pregnancy rate (%) | pregnant (total) | Pregnancy rate (%) | P value^a |
| Overall                       | 943 (10,318) | 9.2    | 265 (3,015) | 8.8              | 678 (7,303) | 9.3             | .225      |
| Patient age, y                |              |        |              |                  |                |                |           |
| ≤ 35                          | 451 (4,118) | 11.0   | 130 (1,284) | 10.1             | 321 (2,834) | 11.3            | .138      |
| 36–37                         | 193 (1,970) | 9.8    | 46 (502)    | 9.2              | 147 (1,468) | 10.0            | .324      |
| 38–39                         | 178 (1,906) | 10.3   | 45 (529)    | 8.5              | 133 (1,377) | 9.7             | .248      |
| 40–41                         | 100 (1,492) | 6.7    | 37 (431)    | 8.6              | 63 (1,061) | 5.9             | .043      |
| 42–43                         | 21 (832)    | 2.5    | 7 (269)     | 2.6              | 14 (563)    | 2.5             | .544      |
| Ovulation induction agent (%) |              |        |              |                  |                |                |           |
| Natural cycle                 | 696 (7,740) | 9.0    | 191 (2,189) | 8.7              | 505 (5551) | 9.1             | .320      |
| Clomiphene citrate            | 202 (2,139) | 9.5    | 52 (592)    | 8.8              | 150 (1547) | 9.7             | .289      |
| Aromatase inhibitor           | 29 (260)    | 11.2   | 16 (164)    | 9.8              | 13 (96)    | 13.5            | .230      |
| Gonadotropin                  | 13 (155)    | 8.4    | 5 (66)      | 7.6              | 8 (89)     | 9.0             | .497      |
| Cyclofenil (Sexovid)          | 3 (24)      | 12.5   | 1 (4)       | 25.0             | 2 (20)     | 10.0            | .437      |
| Infertility diagnosis (%)     |              |        |              |                  |                |                |           |
| Sexual dysfunction            | 72 (614)    | 11.8   | 27 (172)    | 15.7             | 45 (442)   | 10.2            | .041      |
| Unexplained                   | 745 (8,934) | 8.3    | 212 (2,460) | 8.6              | 533 (5,934) | 9.0             | .313      |
| Cervical factor               | 126 (1,310) | 9.6    | 26 (383)    | 6.8              | 100 (927)  | 10.8            | .015      |

Note: Data presented as n (%) for dichotomous variables, unless specified otherwise. DGC = density-gradient centrifugation; MIGLIS = migration-gravity sedimentation without centrifugation.
^a P values for all factors assessed using Fisher’s exact probability test.

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DNA damage (3, 10). Furthermore, the transition metals reactive oxygen species and cause de novo double-stranded breaks among women 40 to 41 years of age. The physical shearing forces of DGC generate such as iron, copper, and aluminum contained in the colloidal suspension. It takes several minutes to apply the semen in the standing temperature and time will further improve the outcomes of MIGLIS. MIGLIS can also be applied to select good spermatozoa with higher motility and greater fertilization ability for both IVF and ICSI. However, the pregnancy rate in women 40–41 years of age was better after MIGLIS than after DGC, which may imply that use of MIGLIS can achieve better pregnancy rates in older women by selecting sperm without DNA damage. However, the relationship between DNA fragmentation and pregnancy rate is still controversial (2, 5), and we did not evaluate sperm DNA damage after MIGLIS preparation in this study. Further studies are therefore necessary to clarify this relationship, and to compare DNA fragmentation in the sperm prepared by MIGLIS and DGC. These studies could confirm the hypothesis that MIGLIS selects sperm without DNA damage and thus improves the pregnancy rate in older women.

Concerning other sperm preparation methods, it has already been shown that the direct swim-up method, which requires less centrifugation, is inferior to DGC in terms of pregnancy rates (18). Macrofluidic sperm sorting, which also involves a device without DGC, has recently been highlighted (19). This device extracts spermatozoa swimming up through an 8-μm microporous filter and is an effective means of removing harmful substances. In contrast, MIGLIS selects spermatozoa swimming over the edge of the cup, so that it would be effective for selecting good spermatozoa with higher motility. In addition, despite using fewer injected spermatozoa, MIGLIS resulted in a pregnancy rate similar to that achieved with DGC, suggesting that it could select spermatozoa with greater fertilizing ability. MIGLIS may thus be an effective tool not only for IUI but also for IVF or ICSI.

MIGLIS takes longer than DGC to complete sperm preparation. It takes several minutes to apply the semen in the first step; however, the preparation then only needs to stand at room temperature and normal gas phase for 1 hour. It requires less effort than DGC, which requires at least two centrifugations. This would thus reduce the staff time required to prepare sperm, making it feasible in clinics with few andrologists. Furthermore, the current study used PBS as the culture solution; however, the replacement of PBS with a sperm culture solution, the addition of some proteins, and changes to the standing temperature and time will further improve the outcomes of MIGLIS. MIGLIS may thus be applied to select good spermatozoa with higher motility and greater fertilization ability for both IVF and ICSI. However, it should be noted that samples with a motile sperm concentration <10 × 10⁶/mL were subjected to sperm preparation using the small-scale Percoll two-layer method and were excluded from this cohort. Also, sperm prepared with MIGLIS inevitably includes a small amount of seminal plasma in the sperm suspension, and one sperm wash may be required when used for IVF or ICSI. Further studies are therefore needed to determine the optimal way to use MIGLIS for IVF or ICSI.

This study aimed to demonstrate the efficacy of MIGLIS as a new sperm preparation method, with results equivalent to those achieved with the established DGC procedure. A strength of the study was that all IUI procedures were conducted in the same institution under the same treatment policy. In addition, the same catheters and same sperm medium were used for IUI throughout this study, and the only variable was therefore the use of DGC or MIGLIS. Furthermore, we conducted two subanalyses to validate our results and to detect any bias. The results of both confirmed that MIGLIS tended to result in a count, MIGLIS resulted in a pregnancy rate similar to that achieved with DGC overall and a higher pregnancy rate among women 40 to 41 years of age.

Several studies have reported damage to sperm DNA following DGC. The physical shearing forces of DGC generate reactive oxygen species and cause de novo double-stranded DNA damage (3, 10). Furthermore, the transition metals such as iron, copper, and aluminum contained in the colloidal solution used for DGC have an affinity for nucleic acids and thus induce oxidative DNA damage (11). DGC also removes endogenous antioxidants in seminal plasma (12). Damage to sperm DNA by DGC must be taken into account in relation to not only the pregnancy rate but also the potential effects on subsequent offspring. Some reports have suggested that damage to sperm DNA increases the risk of autism, bipolar disorder, and schizophrenia in the resulting offspring, in addition to causing organic abnormalities such as cancer or chondrodysplasia (13, 14). Moreover, DGC was recently reported to increase DNA fragmentation, especially in infertile men, according to terminal deoxynucleotidyl transferase dUTP nick-end labeling assay (2), given that sperm DNA might already be predominantly damaged in men with semen abnormalities (15). Suzuki et al. reported that in 30 samples, the rate of DNA fragmentation detected by Halosperm G2 (Halotech DNA) was significantly lower with MIGLIS compared with DGC and the original semen sample (MIGLIS vs. DGC vs. original semen sample, 1.8% vs. 10.8% vs. 23.6%, respectively) (16).

Damage to sperm DNA via fragmentation is more likely to be a problem in older women because of the reduced DNA repair ability associated with cytoplasmic aging (6). We previously reported that advanced paternal age does not affect pregnancy outcomes (17), but advanced maternal age obviously does. The low pregnancy rate associated with advanced maternal age is caused mainly by unseparated chromosomes.
higher pregnancy rate in women 40–41 years of age, suggesting that it provides a good alternative to DGC.

IUI is a second-line process in the step-up method of infertility treatment, and can be performed even in clinics that do not specialize in infertility treatment. MIGLIS is inexpensive, easy to perform in an outpatient setting, and could extract good sperm for IUI. Furthermore, MIGLIS could select sperm without the DNA damage associated with DGC, as well as selecting sperm with higher motility and greater fertilization ability. MIGLIS may represent a breakthrough device in terms of sperm preparation for IUI, IVF, and ICSI, with the potential to improve the quality of reproductive medicine and contribute to successful pregnancies in many patients.

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