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Original Article

Andrology

Swim-up versus density gradients for sperm preparation in infertile couples undergoing intrauterine insemination: a randomized clinical trial

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ABSTRACT

STUDY QUESTION: What is the effectiveness of swim-up (SU) and density gradients (DG) for sperm preparation in infertile couples undergoing IUI?

SUMMARY ANSWER: In infertile couples undergoing IUI, SU and DG did not result in statistically significant different live birth rates.

WHAT IS KNOWN ALREADY: SU and DG are the two most commonly used techniques for sperm preparation in infertile couples undergoing IUI. In the latest Cochrane review, given the very low quality of available data, the authors were uncertain whether there was a difference in clinical pregnancy rates between the two techniques. Furthermore, live birth rate was not reported in any trial.

STUDY DESIGN, SIZE, DURATION: This open-label, two-centre, randomized clinical trial was conducted at two IVF centres in Vietnam. A sample size of 912 couples was needed to demonstrate a 5% difference between SU and DG (power 0.80, two-sided alpha 5%, loss to follow-up, and cross-over rate 10%). Randomization was performed using a computer-generated random list, with a variable block size of 2, 4, or 6. Assignment to treatment allocation was done via a web portal.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eligible couples included those who were ≥18 years of age, where the husbands' sperm concentration, progressive motility (PM) rate, and total PM sperm count before sperm preparation were $\geq 5 \times 10^6$/ml, ≥32%, and $>5 \times 10^6$ (according to the WHO 2010 criteria), respectively. Couples using frozen semen, or couples where the husband's semen was hyperviscous, were not included. On the day of IUI, participants were randomized (1:1 ratio) to undergo either SU or DG. Sperm preparation was performed within 1 h after ejaculation. IUI was performed once at 36–40 h after hCG trigger. Primary outcome was live birth after the first IUI cycle.

MAIN RESULTS AND THE ROLE OF CHANCE: Between 7 August 2020 and 29 October 2022, we randomized 456 couples to SU and 456 couples to DG. Live birth after the first IUI cycle occurred in 55 (12.1%) couples in the SU group and 71 (15.7%) couples in the DG group (relative risk 0.77; 95% CI 0.56 to 1.07). There were no statistically significant differences between the two groups in terms of other pregnancy outcomes as well as obstetrics and perinatal outcomes.

LIMITATIONS, REASONS FOR CAUTION: The main limitation of the study was its open-label design, due to the nature of the interventions.

WIDER IMPLICATIONS OF THE FINDINGS: In infertile couples undergoing IUI, SU and DG can both be used for sperm preparation. The decision on which to use might depend more on practical factors such as processing time and how easy it is to standardize the method.

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Introduction

IUI is considered as a simple, minimally invasive, and cost-effective treatment option for infertility due to being performed without expensive infrastructure (Bahadur *et al.*, 2020). Despite a lack of IUI registration in most countries, IUI is considered as the most widely used method for infertility treatment worldwide (Cohlen *et al.*, 2018).

One of the crucial steps in IUI is sperm preparation, a process that aims to isolate highly motile and morphologically normal sperm while eliminating elements detrimental to fertilization and capacitation (Abdelkader and Yeh, 2009; WHO, 2010). Swim-up (SU) and density gradients (DG) have emerged as the most popular sperm processing techniques among the various methods available. SU involves the separation of motile from immotile sperm based on their ability to swim out of seminal plasma into culture medium, elevating the overall quality of the selected sperm fraction. DG selectively isolates high-quality sperm based on their density using centrifugation, enriching for good-quality separation from debris and other cell types (WHO, 2010).

The ideal sperm separation technique should (i) be efficient in isolating as much motile spermatozoa with high capacity in fertilization as possible; (ii) not cause sperm damage or non-physiological alterations of the separated sperm cells; and (iii) be quick, easy, and cost-effective (Henkel and Schill, 2003). In the latest Cochrane review, which includes six randomized clinical trials (RCTs), it was uncertain whether SU or DG was superior, due to the very low quality of evidence. Moreover, no trials reported on live birth as the primary outcome (Boomsma *et al.*, 2019). In view of this lack of evidence, we performed an RCT comparing SU versus DG for sperm preparation in infertile couples undergoing IUI, with live birth as the primary endpoint.

Materials and methods

Study design and participants

This two-centre, open-label, RCT was conducted at two IVF centres in Ho Chi Minh City, Vietnam (IVFMD, My Duc Hospital; IVFMD Phu Nhuan, My Duc Phu Nhuan Hospital). The andrology laboratories, where semen samples were processed, at these two centres have been following the WHO laboratory manual for the examination and processing of human semen (WHO, 2010) since 2010 (IVFMD) and 2015 (IVFMD Phu Nhuan). These laboratories are also certified by the Ho Chi Minh City Society for Reproductive Medicine, Vietnam.

The protocol was approved by the institutional ethics committee at My Duc Hospital (03/20/ĐĐ-BVMĐ) and registered at ClinicalTrials.gov (NCT04477356). The study was conducted according to Good Clinical Practice and Declaration of Helsinki 2002 principles, including oversight by an independent Data Safety Monitoring Committee (DSMC). Written informed consent was obtained from all couples prior to randomization.

Eligible couples included those who were ≥18 years of age, where the husbands' sperm concentration, progressive motility (PM) rate, and total PM sperm count before sperm preparation were ≥5 × 10^6/ml, ≥32%, and >5 × 10^6 (according to the WHO 2010 criteria), respectively. Couples using frozen semen or couples where the husband's semen was hyperviscous were not eligible.

Randomization and blinding

Potentially eligible couples were given written information about the study on Day 2 or Day 3 of the menstrual cycle, when ovarian stimulation was started. Screening for eligibility was performed by treating physicians on the day of IUI, after the semen had been obtained and before sperm preparation was performed. Couples who fulfilled the eligibility criteria and who had been counselled before were formally invited to participate in the study. After a subsequent discussion with investigators, if the couples agreed to participate, they were asked to sign the informed consent form. After signing the written informed consent form, couples were randomized in a 1:1 ratio to receive either SU or DG, using block randomization with a variable block size of 2, 4, or 6. The computer-generated random list was prepared by an independent statistician who had no other involvement in the study. Assignment to treatment allocation was done via a web portal hosted by HOPE Research Centre, Vietnam. Couples and clinicians performing IUI were unaware of the treatment allocation, while laboratory personnels due to the type of interventions, were not blinded.

Procedures

Ovarian stimulation

For couples with a regular cycle, ovarian stimulation was performed by using hMG (IVF-M 75 IU, LG Life Science, Korea). Couples with ovulation disorders were offered aromatase inhibitor (AI) (Femara® 2.5 mg, Novartis, Switzerland) as the first-line treatment (Teede *et al.*, 2023). However, the choice of agent was also based on the couple's decision. For couples receiving hMG, 75 IU hMG (IM) was started on Day 2 or Day 3 of the menstrual cycle. Follicular development was monitored by transvaginal ultrasound starting from Day 6 of hMG use. When at least one leading follicle reached 18 mm, hCG (IVF-C 5000 IU, LG Life Science®, Korea) was administered.

For couples receiving AI, 5 mg of AI was administered daily for 5 days, starting on any day between Day 2 and Day 5 of the menstrual cycle. Follicular monitoring was performed by transvaginal ultrasound 3 days after the last dose of AI. Another ultrasound was scheduled in the next 2–3 days if the follicle diameter was <12 mm. At 7 days from the last dose of AI, if the average follicle diameter was still <12 mm, hMG was added with a dose of 75 IU. When at least one leading follicle reached 18 mm, hCG was administered.

In both regimens, IUI was performed at 36–38 h after hCG injection. Cycle was cancelled or converted to IVF or IVM if there were more than three follicles ≥14 mm on the day of hCG administration.

Sperm preparation

On the day of IUI, a semen sample was obtained from the husband through masturbation after 2–5 days of abstinence, and it was allowed to liquefy for up to 60 min. Sperm preparation was performed within 1 h after ejaculation.

In the SU group, each 1 ml of semen was gently layered underneath 1.2 ml of Ferticult® Flushing media (Fertipro® NV, Beernem, Belgium) in a 14 round bottom tube (NUNC®, Thermo Fisher Scientific, Denmark). The tube was then inclined at an angle of about 45 degrees and incubated at 37°C for 45–60 min. After incubation, the tube was set to an upright position, and 1 ml of the upper layer was gently aspirated with a sterile Pasteur pipette. This was diluted with 2 ml of Ferticult® Flushing media in a 5 ml disposable plastic centrifuge tube (NUNC®, Thermo Fisher Scientific, Denmark) and centrifuged at 244*g* (1200 rpm) for 10 min. After centrifugation, the supernatant was discarded, leaving 0.3 ml pellet remaining, which was ready for IUI and the assessment of concentration and motility.

In the DG group, gradient medium in 90% (Sil-Select Plus lower layer) and 45% (Sil-Select Plus upper layer) (Sil-Select Plus gradient®, Fertipro NV, Beernem, Belgium), 1.5 ml each, was gently added in a 11 ml conical centrifuge tube (NUNC®, Thermo Fisher Scientific, Denmark). Then, approximately 1–1.5 ml of liquefied semen was placed on top of the upper layer. The sample was centrifuged at 244*g* for 15 min. After centrifugation, the supernatant was removed, and the pellet was suspended in a 5 ml round disposable plastic centrifuge tube with 3 ml Ferticult Flushing media. The sample was centrifuged again at 244*g* for 10 min. The pellet was washed again with 2 ml of Ferticult® Flushing media by centrifugation at 244*g* for 10 min. After that, the supernatant was discarded, leaving a 0.3 ml pellet, which was ready for IUI and the assessment of concentration and motility.

All procedures were conducted in the andrology laboratory, where only two to three senior embryologists with a minimum of 5 years of experience were involved, strictly adhering to standard operating procedures (SOPs). Prior to the initiation of the study, a quality assurance system has been implemented in both centres to meet certification requirements for processes in the andrology laboratory. This system includes: (i) daily checks of equipment/instrument functionality and semi-annual calibration and maintenance; (ii) quarterly audits to identify non-conformity to standard operation procedures or deviations from key performance indicator thresholds, such as turnaround time, sperm recovery rate, clinical pregnancy rate, and inter-technician agreement on semen analysis; and (iii) annual in-house staff competency and proficiency testing in semen analysis and processing.

In both groups, within 15–60 min after preparation, the post-washed sample was used for insemination in the woman's uterus via a soft catheter Gynetics® (Gynétics, Belgium). Women were asked to take bed rest for about 15 min before discharge.

Luteal phase support consisted of 200 mg micronized progesterone twice daily (Cyclogest® 200 mg, Actavis, UK), starting from the day of insemination until the pregnancy test was performed. In case of pregnancy, luteal phase support was continued up to 7 weeks' gestation. Ultrasonography was performed at gestational weeks 7 and 12. Follow-up and management of pregnant people were performed per routine clinical practice during their gestational period.

Outcomes

The primary outcome was live birth after the first IUI cycle. Live birth was defined as the complete expulsion or extraction from the uterus of a product of fertilization after 24 completed weeks of gestational age, which, after such separation, breathes or shows any other evidence of life, such as heartbeat, umbilical cord pulsation, or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached. Twin delivery was considered as a single count.

Secondary outcomes were total motile sperm count (TMSC) after sperm preparation, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, ectopic pregnancy, multiple pregnancy, vanishing twin and miscarriage, gestational age at delivery, preterm delivery, spontaneous preterm delivery, iatrogenic preterm delivery before weeks 24, 28, 32, or 37 of gestation, stillbirth, birthweight, low birthweight, very low birthweight, high birthweight, very high birthweight, 1- and 5-min Apgar score, admission to neonatal intensive care unit (NICU) and congenital abnormalities. Recovery rate, defined as the number of motile sperm after preparation in relation to the total number of motile sperm before preparation, was added post-hoc as a secondary outcome.

Statistical analysis

Prior to the study, the live birth rate after IUI using DG for sperm preparation at IVFMD was 13.4% per cycle. Assuming that the clinically relevant difference in live birth rate between the two methods was 5%, we needed 912 couples (456 per arm) (80% power, two-sided alpha of 0.05, loss to follow-up, and cross-over rate 10%). The 5% difference was chosen as a clinically relevant difference based on the simplicity, and minimal invasiveness of the procedures, as well as to ensure a reliable statistical power. We planned an interim analysis after enrolling the first 456 participants, using ongoing pregnancy as endpoint. A two-sided significance test with the Haybittle–Peto spending function was used, with a type I error rate of 5% and stopping criteria of $P < 0.001$ (Z-alpha = 3.29) for differences in ongoing pregnancy rates. After 456 couples had been enrolled (6 August 2021), ongoing pregnancy results were available in 192 couples in the SU group and 188 couples in the DG group. The interim analysis did not show a reason to stop the study early.

The primary statistical analysis was conducted on an intention-to-treat basis using the R statistical program (R Foundation, Vienna, Austria, version 4.3.0). Baseline data were presented using descriptive statistics (mean and SD for normally distributed variables or median and interquartile range for skewed variables). Categorical data were presented as number (%). The primary outcome, live birth rate after the first IUI cycle, was compared between groups by calculating the relative risk (RR) and the associated 95% CI. Between-group differences in secondary endpoints were analysed using parametric methods (normally distributed data), nonparametric methods (skewed data), or Fisher's exact test (categorical variables) and were reported as RR and 95% CIs. The absolute differences and the 95% CI of skewed data were calculated using the Hodges–Lehman method. Pre-planned subgroup analysis was performed to examine the live birth rate stratified by IUI indications and post-washed TMSC, which was divided into five groups: <1, ≥1 and <5, ≥5 and <10, ≥10 and <20, and ≥20 million. Live birth rates and post-washed TMSC stratified by different embryologists handling the samples were also performed as post-hoc analysis. A *P*-value of <0.05 was considered to indicate statistically significant differences.

Results

Between 7 August 2020 and 29 October 2022, a total of 1223 couples were assessed for eligibility. Of the 965 eligible couples, 53 couples declined to participate. Therefore, 912 couples were randomized to SU (n = 456) or DG (n = 456) (Fig. 1). Baseline characteristics were

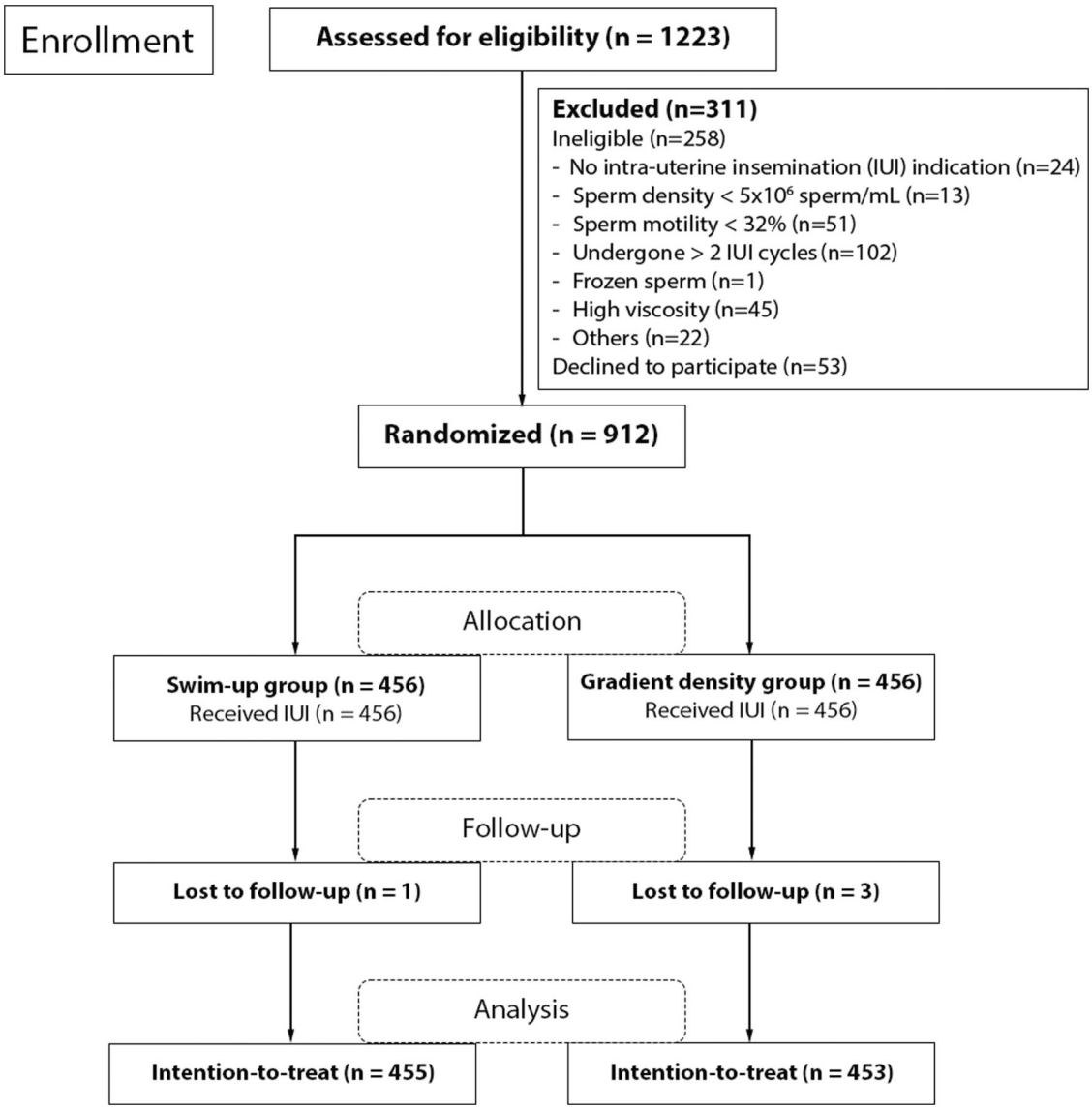

Figure 1. **Trial profile.**

comparable between the two groups (Table 1). Follow-up was completed on 22 July 2023, with four couples lost to follow-up after the confirmation of ongoing pregnancy (Supplementary Table S1). Therefore, a total of 908 couples remained in the final analysis.

The primary outcome, live birth after the first IUI cycle, occurred in 55 (12.1%) couples in the SU group compared to 71 (15.7%) couples in the DG group (RR 0.77, 95% CI 0.56 to 1.07, $P = 0.16$; absolute risk difference −3.6%, 95% CI −8.08 to 0.91) (Table 2). There were lower rates of clinical pregnancy and ongoing pregnancy in couples treated by SU compared to those treated by DG. However, these differences were not statistically significant. The median post-washed TMSC in the SU and DG groups showed no significant difference (Table 2). Obstetrics and neonatal outcomes were also comparable between the two groups (Table 3). Pre-planned subgroup analysis showed that the live birth rate was not materially affected by the IUI indications or the post-washed TMSC (<1, ≥1 and <5, ≥5 and <10, ≥10 and <20, and ≥20 million) (Supplementary Table S2). In a post-hoc analysis, the sperm recovery rate was significantly lower in the SU group compared to that in the DG group (absolute difference −1.5, 95% CI −2.6 to −0.5; Table 2). Live birth rates and post-washed TMSC were also comparable between the two groups, regardless of the different embryologists handling the samples (Supplementary Tables S3 and S4).

Discussion

In this RCT, we found that DG resulted in a 3.6% increase in live birth compared to SU. However, this difference was not statistically significant. The same trend in clinical and ongoing pregnancy was found. Obstetrics and neonatal outcomes were also comparable between the two groups.

The aim of sperm preparation is to separate normal sperm from the debris of the ejaculate and, in the case of IUI, to yield as many normal, motile, and high-fertilization-capacity spermatozoa as possible. The SU method is based on the ability of the spermatozoa to swim. In this procedure, the motile spermatozoa swim up to the culture medium layered over the liquified semen. For DG, centrifugation is used to separate spermatozoa according to their density. By this way, the motile, functional spermatozoa can be selected in the solution with the highest concentration (Henkel and Schill, 2003). Because of the fundamental differences

Table 1. Baseline characteristics.

	Swim-up (n = 456)	Density gradients (n = 456)
Female age (years)	31.2 ± 4.2	31.3 ± 3.8
Male age (years)	33.7 ± 5.1	33.6 ± 4.5
Female BMI (kg/m^2)	21.2 ± 2.5	21.5 ± 2.7
Duration of infertility (months)	24.0 [12.0; 36.0]	24.0 [12.0; 36.0]
Female anti-Müllerian hormone (ng/ml)	5.1 ± 3.6	5.3 ± 3.7
IUI indication		
Unexplained infertility	155 (34.0)	156 (34.2)
Male factor	44 (9.6)	30 (6.6)
Tubal factor	14 (3.1)	11 (2.4)
Ovulation disorders	153 (33.6)	163 (35.7)
Others	90 (19.7)	96 (21.1)
Primary infertility	288 (63.2)	308 (67.5)
Number of previous IUI attempts		
0	418 (91.7)	416 (91.2)
1	38 (8.3)	40 (8.8)
Type of ovulation induction		
hMG	278 (60.9)	280 (61.4)
AI	144 (31.6)	138 (30.3)
AI + hMG	34 (7.5)	38 (8.3)
Endometrium thickness (mm)	10.3 ± 2.0	10.4 ± 2.0
Days of abstinence (days)	2.4 ± 1.1	2.4 ± 1.9
Semen volume (ml)	2.6 ± 1.3	2.5 ± 1.1
Sperm concentration (million/ml)	39.5 [25.0; 55.2]	38.0 [25.0; 55.0]
Total sperm count (million)	86.7 [54.3; 137.9]	86.0 [52.4; 136.5]
Sperm motility (%)	44.7 ± 8.4	44.2 ± 9.1
Total motile sperm count (million)	38.4 [22.3; 63.1]	37.8 [20.8; 63.7]
Sperm with normal morphology (%)*	2.0 [1.0; 3.0]	2.0 [1.0; 3.0]

Plus–minus values are means ± SD; median [IQR]; N (%).
* Data from samples obtained during the first consultation.
AI: aromatase inhibitor; IQR: interquartile range.

in these two methods, one might expect that there would also be a difference in the treatment outcomes. In fact, this has been a matter of debate for many years (Dodson et al., 1998; Morshedi et al., 2003; Karamahmutoglu et al., 2014; Butt and Chohan, 2016).

Our trial is the first to report on live birth within a robust design and an adequate sample size. Although there was no significant difference in all pre-defined outcomes, DG resulted in a 3.6% increase in live birth rate. This suggests that the chance of having a live birth when using DG could be 20% higher than that when using SU. While the CI mostly aligns with an improved chance of live birth with DG, given the simplicity and minimal invasiveness of the procedure, this difference could be clinically significant. However, further research is needed to confirm this finding. Our post-hoc analysis showed that DG resulted in a significantly higher recovery rate compared to SU. While caution is needed in the interpretation of a post-hoc analysis, this finding aligns with the latest Cochrane review (Boomsma et al., 2019) and with the WHO guidelines (WHO, 2010, 2021). While SU method selects highly motile sperm and may be preferable for good-quality samples, the DG method can recover a greater number of motile sperm and may be suitable for all sample types (WHO, 2010, 2021). Consequently, DG appears to be the preferred approach for sperm preparation prior to IUI, despite our findings not having revealed a significant difference between the two methods.

Post-washed TMSC has been suggested as one of the important factors affecting the success of IUI. However, while some studies indicated an association between ongoing pregnancy and post-washed TMSC (Ok et al., 2013; Hansen et al., 2020; Muthigi et al., 2021), others showed that post-washed TMSC had no effect on pregnancy outcomes (Lemmens et al., 2016; Findeklee et al., 2020; Liu et al., 2021). In this study, we also found that live birth rates were not materially affected by the IUI indications and the post-washed TMSC in both SU and DG groups. Additionally, the median post-washed TMSC was comparable between the two groups, albeit there was a non-statistically significant 3.6% higher live birth rate in the DG group. The rationales behind these findings need to be elucidated in future research.

There are limited data on the safety profile of sperm preparation techniques. There are so far no adverse events reported, either with SU or DG, as long as the handling strictly adheres to the WHO guidelines (Cohlen et al., 2018). DNA fragmentation is the most frequently cited potential side effect with sperm preparation (Baldi et al., 2020). However, most data are derived from observational studies (Balasuriya et al., 2014; Zhao et al., 2016; Raad et al., 2021; Le et al., 2022). In an RCT involving a limited number of infertile men (n = 65), the authors showed that SU significantly decreases sperm DNA fragmentation (SDF) rate in both unexplained and mild male factor infertility (Oguz et al., 2018). Future studies, in a randomized setting and with adequately large sample size, should investigate the relationship between sperm preparation methods and DNA integrity to provide a more holistic view of their implications for clinical practice.

In addition to effectiveness and safety, the choice between SU or DG for couples undergoing IUI would also depend on cost considerations. While SU and DG share many similarities in equipment and resources, operational costs may vary due to factors like processing time and personnel training. Unfortunately, there is currently no report on the cost-effectiveness of these two interventions. Therefore, future research should prioritize evaluating the cost-effectiveness of both techniques, alongside clinical outcomes.

Strengths of our trial include the use of live birth as primary outcome, its randomized design, the adequately large sample size, and the low loss of follow-up as well as a limited number of embryologists involved in the SU or DG process. All involved embryologists had at least 5 years of experience with the procedures. However, some limitations need to be considered. First, the lack of blinding to laboratory personnel, due to the nature of the interventions, could influence the handling and preparation of sperm samples. However, this is a common limitation in many other studies involving interventions in the laboratory (Karamahmutoglu et al., 2014; Munne et al., 2019; Dang et al., 2021; Wang et al., 2024). In this trial, we tried to minimize this potential bias by strictly adhering to the SOPs and ensuring similar patient management and protocolized follow-up in both groups. Also, the use of live birth as primary outcome helped to prevent ascertainment bias. Second, the trial was conducted at two IVF centres in Ho Chi Minh City, Vietnam, with most couples being young, having an adequate ovarian reserve, and low BMI, and 90% of couples were in their first treatment attempt. Additionally, our trial excludes couples with lower sperm count or motility issues, which might compromise the generalizability of our findings. Third, the study only focuses on outcomes after the first IUI cycle, while the potential cumulative effects over multiple cycles could be of interest in clinical practice. The cost-effectiveness of the two techniques, which is important for clinical decision-making and resource allocation in reproductive medicine, was not evaluated. Four, this study does not report any adverse events associated with either SU or DG. This is in agreement with the wider field, as to the best of the authors' knowledge, so far no adverse events have been reported following sperm preparation, either with SU or DG, as long as the handling was strictly adhered to WHO guidelines (WHO, 2010, 2021). Fifth,

Table 2. Fertility outcomes.

	Swim-up (n = 455)	Density gradients (n = 453)	Absolute difference (95% CI)	Relative risk (95% CI)	P-values
Primary outcome					
Live birth (%)	55 (12.1)	71 (15.7)	−3.6 (−8.08 to 0.91)	0.77 (0.56 to 1.07)	0.16
Secondary outcomes					
Biochemical pregnancy (%)	85 (18.7)	101 (22.3)	−3.6 (−8.86 to 1.63)	0.84 (0.65 to 1.08)	0.86
Clinical pregnancy (%)	64 (14.1)	80 (17.7)	−3.6 (−8.34 to 1.15)	0.8 (0.59 to 1.08)	0.17
Ongoing pregnancy (%)	59 (13.0)	73 (16.1)	−3.1 (−7.73 to 1.43)	0.81 (0.59 to 1.11)	0.17
Ectopic pregnancy (%)	3 (0.7)	5 (1.1)	−0.4 (−1.66 to 0.77)	0.6 (0.14 to 2.48)	0.73
Multiple pregnancy (%)	7 (1.5)	9 (2.0)	−0.5 (−2.16 to 1.26)	0.77 (0.29 to 2.06)	0.50
Twins	6 (1.3)	6 (1.3)	0 (−1.49 to 1.48)	1.0 (0.33 to 3.1)	0.95
Higher orders	1 (0.2)	3 (0.7)	−0.5 (−1.30 to 0.42)	0.33 (0.03 to 3.18)	0.31
Foetal reduction (%)	2 (0.4)	2 (0.4)	0 (−0.86 to 0.86)	1.0 (0.14 to 7.03)	0.95
Vanishing twins (%)	2 (0.4)	1 (0.2)	0.2 (−0.53 to 0.96)	2.0 (0.18 to 21.86)	0.95
Miscarriage	4 (0.9)	3 (0.7)	0.2 (−0.92 to 1.35)	1.33 (0.3 to 5.89)	0.71
Post-washed total motile sperm count (millions)	9.0 [5.0; 13.5]	9.0 [5.0; 15.6]	0 (−1.2 to 1.04)	–	0.50
Sperm recovery rate* (%)	23.7 ± 8.8	25.1 ± 7.8	−1.5 (−2.6 to −0.5)	–	0.008

* Post-hoc analysis; plus–minus values are means ± SD; median [IQR]; N (%).
IQR: interquartile range.

Table 3. Obstetrics and perinatal outcomes.

	Swim-up (n = 455)	Density gradients (n = 453)	Absolute difference (95% CI)	Relative risk (95% CI)	P-values
Obstetrics outcomes					
Gestational age at delivery (weeks)	38.4 ± 1.3	38.0 ± 1.7	0.4 (−0.2 to 1.1)	–	0.17
Stillbirth	0	0	–	–	–
Premature birth					
Delivery <24 weeks	0	0	–	–	–
Delivery 24–<28 weeks	0	0	–	–	–
Delivery 28–<32 weeks	0	1 (0.2)	−0.2 (−0.65 to 0.21)	–	–
Delivery 32–<37 weeks	6 (1.3)	9 (2.0)	−0.7 (−2.33 to 0.99)	0.66 (0.24 to 1.85)	0.43
Spontaneous delivery <24 weeks	0	0	–	–	–
Spontaneous delivery 24–<28 weeks	0	0	–	–	–
Spontaneous delivery 28–<32 weeks	0	1 (0.2)	−0.2 (−0.65 to 0.21)	–	–
Spontaneous delivery 32–<37 weeks	5 (1.1)	9 (2.0)	−0.9 (−2.49 to 0.71)	0.55 (0.19 to 1.64)	0.28
Iatrogenic delivery <24 weeks	0	0	–	–	–
Iatrogenic delivery 24–<28 weeks	0	0	–	–	–
Iatrogenic delivery 28–<32 weeks	0	0	–	–	–
Iatrogenic delivery 32–<37 weeks	1 (0.2)	0	0.2 (−0.21 to 0.65)	–	–
Neonatal outcomes	N = 58	N = 76			
Singleton	52 (89.7)	66 (86.8)	2.9 (−8.10 to 13.73)	–	–
Twins	6 (10.3)	10 (13.2)	−2.9 (−13.73 to 8.10)	–	–
Birthweight (g)					
Singleton	3164.4 ± 414.0	3029.2 ± 436.4	135.2 (−21.2 to 291.5)	–	0.09
Twins	2233.3 ± 250.3	2405.0 ± 476.4	−171.7 (−562.4 to 219.0)	–	0.36
Low birthweight (%)*	3/52 (5.8)	6/66 (9.1)	–	0.63 (0.17 to 2.42)	0.51
Very low birthweight (%)*	0	0	–	–	–
High birthweight (%)*	1/52 (1.9)	0	1.92 (−1.81 to 5.66)	–	–
Very high birthweight (%)*	1/52 (1.9)	0	1.92 (−1.81 to 5.66)	–	–
1-min Apgar score	8.0 [8.0; 8.0]	8.0 [8.0; 8.0]	0 (−0.4 to 0.4)	–	0.95
5-min Apgar score	9.0 [9.0; 9.0]	9.0 [9.0; 9.0]	0.1 (−0.2 to 0.5)	–	0.42

Plus–minus values are means ± SD; median [IQR]; N (%).
* Calculated on the population of singleton.
IQR: interquartile range.

DNA integrity, which is often cited as a potential side effect of sperm preparation, was not evaluated in our trial. While some evidence suggests that SDF may be a potential predictor of pregnancy outcome after IUI (Chen *et al.*, 2019), the ideal method to measure DNA fragmentation and its optimal thresholds are still to be determined. Moreover, at present, limited data exist concerning the clinical utility of medical and surgical interventions to alleviate SDF (Esteves *et al.*, 2020). Sixth, although evidence on the interval between preparation and insemination is inconclusive (Kuru Pekcan *et al.*, 2018; Punjabi *et al.*, 2021; Statema-Lohmeijer *et al.*, 2023), the broad interval in our study (15–60 min) might introduce variability in the sperm quality. Last, the wide CI in the primary outcome suggests that our trial was powered to detect a 5% difference but could not rule out a smaller difference, which might be preferred in some other settings.

In conclusion, in infertile couples undergoing IUI, SU and DG did not result in statistically significantly different live birth rates. The decision on which to use might depend more on practical factors such as processing time and how easy it is to standardize the method.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

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Authors' roles

The study was designed by T.M.H., V.Q.D., T.N.D.D., T.K.L., and B.W.M. Statistical analysis was done by T.D.P., A.T.L.V., and D.L.N. The first draft of the manuscript was prepared by T.N.D.D., T.K.L., V.Q.D., and B.W.M., who had unrestricted access to the data. The manuscript was reviewed and edited by all the authors. All authors decided to submit the manuscript for publication and assume responsibility for the accuracy and completeness of the analyses and the fidelity of this report to the trial protocol.

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Conflict of interest

B.W.M. is supported by a NHMRC Investigator grant (GNT1176437), reports consultancy, travel support, and research funding from Merck and consultancy for Organon and Norgine, and holds stock from ObsEva. L.N.V. has received grant, speaker, and conference fees from Merck Sharpe and Dohme, and grant, speaker, conference, and scientific board fees from Ferring. T.M.H. has received grant, speaker, and conference fees from Merck Sharpe and Dohme, and grant, speaker, conference, and scientific board fees from Ferring. Other authors have no conflicts of interest to declare.

References

Abdelkader AM, Yeh J. The potential use of intrauterine insemination as a basic option for infertility: a review for technology-limited medical settings. *Obstet Gynecol Int* 2009;**2009**:584837.

Bahadur G, Homburg R, Bosmans JE, Huirne JAF, Hinstridge P, Jayaprakasan K, Racich P, Alam R, Karapanos I, Illahibuccus A et al Observational retrospective study of UK national success, risks and costs for 319,105 IVF/ICSI and 30,669 IUI treatment cycles. *BMJ Open* 2020;**10**:e034566.

Balasuriya A, Serhal P, Doshi A, Harper JC. Processes involved in assisted reproduction technologies significantly increase sperm DNA fragmentation and phosphatidylserine translocation. *Andrologia* 2014;**46**:86–97.

Baldi E, Tamburrino L, Muratori M, Degl'Innocenti S, Marchiani S. Adverse effects of in vitro manipulation of spermatozoa. *Anim Reprod Sci* 2020;**220**:106314.

Boomsma CM, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. *Cochrane Database Syst Rev* 2019;**10**:CD004507.

Butt F, Chohan MA. Comparative efficacy of density gradient and swim-up methods of semen preparation in intrauterine insemination cycles. *J Pak Med Assoc* 2016;**66**:932–937.

Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. *Reprod Toxicol* 2019;**86**:50–55.

Cohlen B, Bijkerk A, Van der Poel S, Ombelet W. IUI: review and systematic assessment of the evidence that supports global recommendations. *Hum Reprod Update* 2018;**24**:300–319.

Dang VQ, Vuong LN, Luu TM, Pham TD, Ho TM, Ha AN, Truong BT, Phan AK, Nguyen DP, Pham TN et al Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial. *Lancet* 2021;**397**:1554–1563.

Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. *Fertil Steril* 1998;**70**:574–575.

Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. *Andrology* 2020;**8**:53–81.

Findeklee S, Radosa JC, Radosa MP, Hammadeh ME. Correlation between total sperm count and sperm motility and pregnancy rate in couples undergoing intrauterine insemination. *Sci Rep* 2020;**10**:7555.

Hansen KR, Peck JD, Coward RM, Wild RA, Trussell JC, Krawetz SA, Diamond MP, Legro RS, Coutifaris C, Alvero R et al Intrauterine insemination performance characteristics and post-processing total motile sperm count in relation to live birth for couples with unexplained infertility in a randomised, multicentre clinical trial. *Hum Reprod* 2020;**35**:1296–1305.

Henkel RR, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol* 2003;**1**:108.

Karamahmutoglu H, Erdem A, Erdem M, Mutlu MF, Bozkurt N, Oktem M, Ercan DD, Gumuslu S. The gradient technique improves success rates in intrauterine insemination cycles of unexplained subfertile couples when compared to swim up technique; a prospective randomized study. *J Assist Reprod Genet* 2014;**31**:1139–1145.

Kuru Pekcan M, Kokanalı D, Kokanalı K, Taşçı Y. Effect of time intervals from the end of sperm collection to intrauterine insemination on the pregnancy rates in controlled ovarian hyperstimulation-intrauterine insemination cycles. *J Gynecol Obstet Hum Reprod* 2018;**47**:561–564.

Le MT, Dang HNT, Nguyen TV, Nguyen TTT, Nguyen QHV, Cao NT. Effects of sperm preparation techniques on sperm survivability and DNA fragmentation. *J Int Med Res* 2022;**50**:3000605221097492.

Lemmens L, Kos S, Beijer C, Brinkman JW, van der Horst FA, van den Hoven L, Kieslinger DC, van Trooyen-van Vrouwerff NJ, Wolthuis A, Hendriks JC, et al; Semen Section of the Dutch Foundation for Quality Assessment in Medical Laboratories. Predictive value of sperm morphology and progressively motile sperm count for pregnancy outcomes in intrauterine insemination. *Fertil Steril* 2016;**105**:1462–1468.

Liu S, Chen Z, Wang H, Yang L, Ma C, Sun L. Prewash and postwash total progressively motile sperm counts have poor predictive value for clinical pregnancy after intrauterine insemination. *Int J Gynaecol Obstet* 2021;**153**:476–482.

Morshedi M, Duran HE, Taylor S, Oehninger S. Efficacy and pregnancy outcome of two methods of semen preparation for intrauterine insemination: a prospective randomized study. *Fertil Steril* 2003;**79** Suppl 3:1625–1632.

Munne S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, Silverberg K, Kalista T, Handyside AH, Katz-Jaffe M, et al; STAR Study Group. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed

embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril* 2019;**112**:1071–1079 e1077.

Muthigi A, Jahandideh S, Bishop LA, Naeemi FK, Shipley SK, O'Brien JE, Shin PR, Devine K, Tanrikut C. Clarifying the relationship between total motile sperm counts and intrauterine insemination pregnancy rates. *Fertil Steril* 2021;**115**:1454–1460.

Oguz Y, Guler I, Erdem A, Mutlu MF, Gumuslu S, Oktem M, Bozkurt N, Erdem M. The effect of swim-up and gradient sperm preparation techniques on deoxyribonucleic acid (DNA) fragmentation in subfertile patients. *J Assist Reprod Genet* 2018; **35**:1083–1089.

Ok EK, Doğan OE, Okyay RE, Gülekli B. The effect of post-wash total progressive motile sperm count and semen volume on pregnancy outcomes in intrauterine insemination cycles: a retrospective study. *J Turk Ger Gynecol Assoc* 2013;**14**:142–145.

Punjabi U, Van Mulders H, Van de Velde L, Goovaerts I, Peeters K, Cassauwers W, Lyubetska T, Clasen K, Janssens P, Zemtsova O et al Time intervals between semen production, initiation of analysis, and IUI significantly influence clinical pregnancies and live births. *J Assist Reprod Genet* 2021; **38**:421–428.

Raad G, Bakos HW, Bazzi M, Mourad Y, Fakih F, Shayya S, McHantaf L, Fakih C. Differential impact of four sperm preparation techniques on sperm motility, morphology, DNA fragmentation, acrosome status, oxidative stress, and mitochondrial activity: a prospective study. *Andrology* 2021; **9**:1549–1559.

Statema-Lohmeijer CH, Schats R, Lissenberg-Witte BI, Kostelijk EH, Lambalk CB, Vergouw CG. A short versus a long time interval between semen collection and intrauterine insemination: a randomized controlled clinical trial. *Hum Reprod* 2023;**38**:811–819.

Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT, Costello MF, Boivin J, Redman LM, Boyle JA, et al; International PCOS Network. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Eur J Endocrinol* 2023;**189**:G43–G64.

Wang Y, Li R, Yang R, Zheng D, Zeng L, Lian Y, Zhu Y, Zhao J, Liang X, Li W et al Intracytoplasmic sperm injection versus conventional in-vitro fertilisation for couples with infertility with non-severe male factor: a multicentre, open-label, randomised controlled trial. *Lancet* 2024;**403**:924–934.

WHO. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th edn. Geneva, Switzerland: World Health Organization, 2010.

WHO. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 6th edition. Geneva, Switzerland: World Health Organization, 2021.

Zhao F, Yang Q, Shi S, Luo X, Sun Y. Semen preparation methods and sperm telomere length: density gradient centrifugation versus the swim up procedure. *Sci Rep* 2016;**6**:39051.

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