Received: 23 April 2020 | Revised: 5 August 2020 | Accepted: 18 August 2020

DOI: 10.1111/and.13852

ORIGINAL ARTICLE

First International Journal of Andrology
ANDROLOGIA
WILEY

A methodological validation of an easy one-step swim-out semen preparation procedure for selecting DNA fragmentation-free spermatozoa for ICSI

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Abstract

The main purpose of this methodological paper was to describe a recently designed one-step ICSI semen preparation swim-out method (called swim-ICSI) and to compare its efficacy with our conventional two-step swim-out method for the selection of motile spermatozoa for ICSI with minimal DNA damage. In this observational cohort study, 42 fresh ejaculate sperm samples for ICSI were included to compare the new swim-ICSI with the conventional swim-out. In a sub-analysis ($n = 20$), both in-house designed ICSI preparation methods were compared with a commercial magnetic-activated cell sorting test (MACS®). Sperm DNA fragmentation (SDF), using Halosperm®, was determined at different time points during sperm preparation: on the native sample (a), after density gradient centrifugation (DG) (b), on the motile (A + B) spermatozoa selected with conventional swim-out post-DG (c) and selected with swim-ICSI method post-DG (d). For a subgroup ($n = 20$), SDF was also calculated after MACS (e). The mean SDF significantly reduced after EACH preparation step and reduced to almost zero in the recovered A + B spermatozoa when the semen prepared with DG was further processed for ICSI (swim-ICSI vs. swim-out, $p = .001$). In conclusion, the optimised one-step and fine-tuned swim-ICSI technique shows the possibility to select a population of spermatozoa with almost zero SDF to be used in ICSI treatments.

KEYWORDS

human sperm preparation, ICSI, magnetic cell sorting, sperm DNA fragmentation, swim-out methods

1 | INTRODUCTION

The selection of human spermatozoa prior to ICSI is mainly based on motility and morphology (Troya & Zorrilla, 2015). Although these conventional semen parameters are very informative and related to the quality of the spermatozoa, they cannot completely evaluate certain intrinsic characteristics of spermatozoa (Volpes et al., 2016). Sperm DNA integrity is essential for accurate transmission of paternal genetic information, and normal sperm chromatin structure is important for sperm fertilising ability. Evaluation of sperm DNA damage appears to be a useful parameter to assess male infertility, and non-invasive selection of spermatozoa without DNA fragmentation is highly recommended in fertility treatments (Oseguera-López et al., 2019). The conventional semen parameters (concentration, motility and morphology) do not identify defects in the sperm chromatin structure although certain correlations have been suggested

in the literature (Erenpreiss et al., 2006). Although still controversial, there are studies reporting a correlation between sperm motility and sperm DNA fragmentation (SDF) (Erenpreiss et al., 2006; Palermo et al., 2014; Punjabi et al., 2018; Xie et al., 2018). Sperm DNA damage is described as one of the most important factors contributing to a decrease in sperm motility (Erenpreiss et al., 2006). Therefore, common sperm preparation techniques like density gradient centrifugation and/or swim-up procedures for the selection of motile spermatozoa may result in a selection of highly motile sperm containing lower DNA fragmentation.

Despite the widespread use of these sperm preparation techniques, there is, at the moment, no consensus on which sperm processing method is most suitable for the selection of spermatozoa for ICSI (Volpes et al., 2016). An ideal sperm processing technique should be gentle and one that recovers a highly functional spermatozoon. Semen preparation methods like density gradient centrifugation or swim-up are used for insemination or conventional IVF, however, for ICSI sometimes only a simple sperm wash is used. The rational for this is that only a limited number of spermatozoa are needed to perform ICSI and microscopic evaluation of the spermatozoa by a trained embryologist would be sufficient to select the right spermatozoa. A simple sperm wash separates spermatozoa from the seminal plasma by centrifugation of the spermatozoa in a buffered medium, while sperm density centrifugation separates sperm cells based on their density. A two-layer gradient of a different density (45%, 90%) separates the highly motile and morphological normal spermatozoa from the seminal plasma, the immotile spermatozoa and immature/abnormal spermatozoa who have a slightly lower density. The swim-up or swim-out procedure separates spermatozoa based on their motility and their capability to move through a buffered medium (without centrifugation). These different sperm preparation techniques can be combined with each other to obtain a better selection of spermatozoa.

In our centre, a thorough sperm preparation for ICSI is performed. After gradient centrifugation, spermatozoon is further processed using an in-house swim-out method, which is based on the swim-up principle, for the selection of progressive (A + B) motile spermatozoa for ICSI. Semen is incubated in a medium where it is allowed to swim alongside a certain medium trajectory. The progressive spermatozoa swim faster and further in the trajectory in comparison with the non-progressive or immotile spermatozoa. To our knowledge only the groups of Palini et al. (2016) and De Martin et al. (2017), presented a non-commercialised modification of the classic swim-up procedure, called the micro swim-up and the positive rheotaxis extended droplet (PRED), respectively, directly correlated with the selection of spermatozoa for ICSI. After the separation of the spermatozoa in the swim-out, the progressive motile spermatozoa are then transferred to a new dish in which the ICSI is performed. Our current method is time-consuming, since the incubation of the swim-out takes approximately 2 hr and a transfer to another dish for performing the ICSI is necessary. The main purpose of this study was to compare the efficacy of a newly designed one-step ICSI semen preparation swim-out method (called the swim-ICSI) where the separation of the spermatozoa takes places immediately in the dish where the ICSI is then performed. The spermatozoa are also only allowed to swim for 20–30 min before they arrive in the collection droplet to be selected for ICSI. Because of this time constraint, the separation of the spermatozoa could be substandard in the possibility to have progressive motile sperm containing minimal DNA damage.

In a sub-analysis, these in-house methods were compared with a commercial magnetic-activated cell sorting test (MACS® ART Annexin V, Miltenyi Biotec, 2016). The ultimate goal of our research was to methodologically validate the isolation and selection of viable spermatozoa with good sperm integrity by evaluating the SDF rate after each processing step.

2 | MATERIAL AND METHODS

2.1 | Study design and patient population

In this observational cohort single-centre study, 42 sperm samples from patients undergoing ICSI cycles at the centre for reproductive medicine of the Ghent University Hospital were included. Patients signed informed consent for the use of residual reproductive material for validation studies (Ethical committee approval EC 227-2016/mf). All samples were fresh ejaculate sperm samples.

For this study, two in-house designed swim-out methods for the selection of human spermatozoa for ICSI were compared: the swim-out versus the more recently designed swim-ICSI. In a sub-analysis ($n = 20$ semen samples), these two swim-out methods were compared with a commercial magnetic-activated cell sorting test (MACS® ART Annexin V, Miltenyi Biotec, 2016) for the selection of apoptotic free and therefore more likely SDF-free spermatozoa. Figure 1 shows a detailed description of the study design. Briefly, after semen analysis, all 42 native semen samples were processed using density gradient centrifugation (DG) and split afterwards to be further processed using the ICSI sperm preparation methods: conventional swim-out ($n = 42$), swim-ICSI ($n = 42$) and MACS® ($n = 20$). The SDF rate was determined at different time points during the preparation of the samples ($n = 42$): on the native sample before preparation (a), after 45/90 density gradient centrifugation (b), on the motile (A + B) spermatozoa selected with the conventional swim-out post-density gradient centrifugation (c), on the motile (A + B) spermatozoa selected with the new swim-ICSI method post-density gradient centrifugation (d) and for a subgroup of 20 samples on the Annexin V-negative spermatozoa (e).

2.2 | Semen analysis

After liquefaction on a heated plate at 37°C for 20 min, the volume of all semen samples was measured and conventional semen parameters (concentration and motility) were determined with the Sperm Class Analyzer® (SCA) system (Microptic). The reference values of

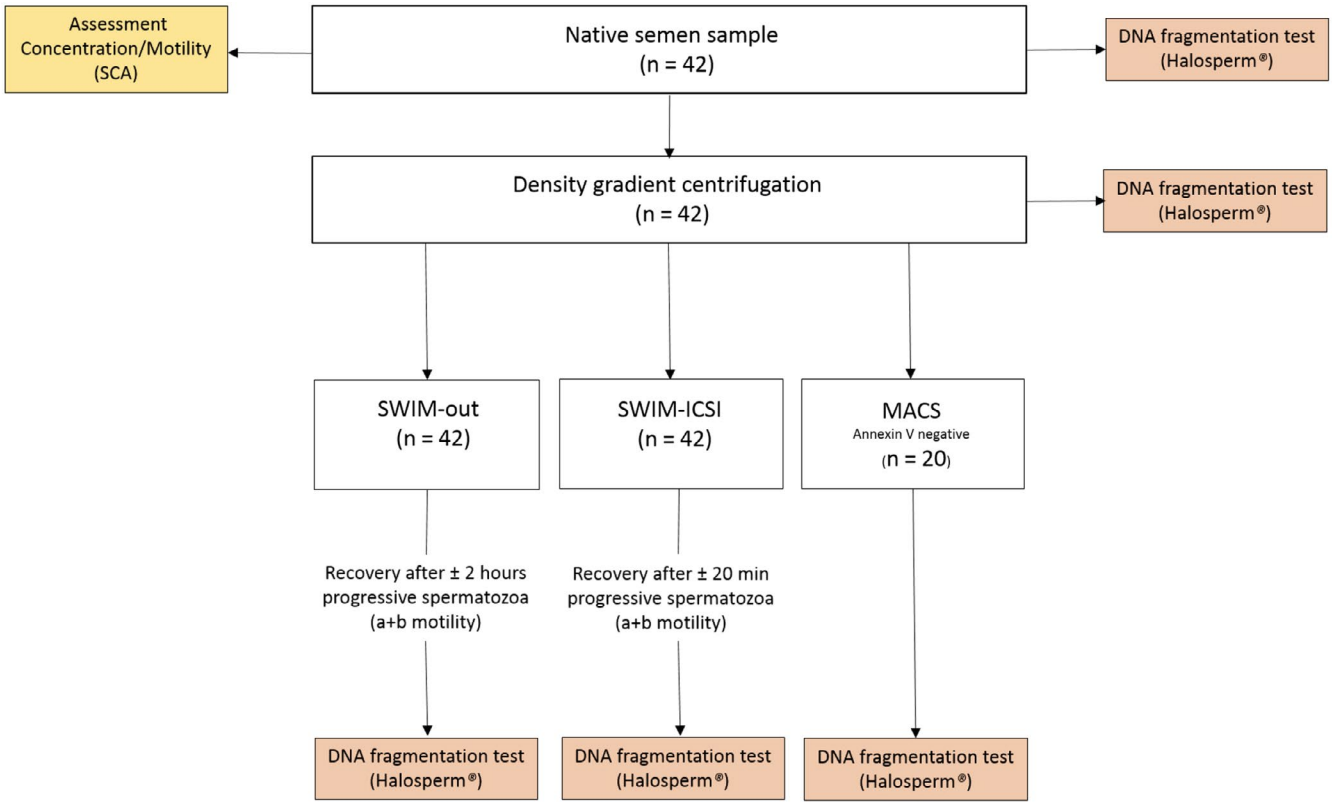

FIGURE 1 Flowchart study design. MACS®, Magnetic Cell Sorting; SCA, Sperm Class Analyzer® system

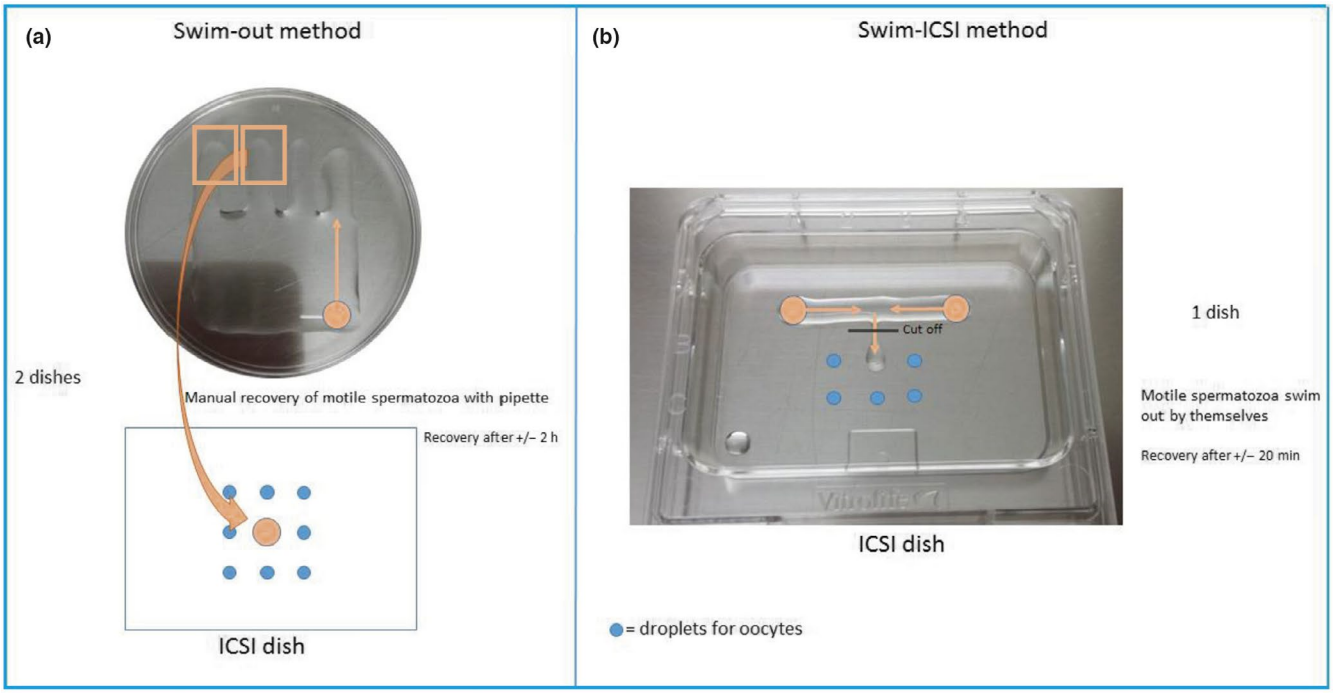

FIGURE 2 Methodological design of in-house designed ICSI preparation methods: conventional swim-out (a) and the new Swim-ICSI (b). The point of loading the sperm sample is indicated with the orange circle, and the direction of sperm movement is indicated with the arrow. The point where the spermatozoa were collected in the swim-out (a) is indicated with a square. The blue circles indicated droplets where oocytes are present for subsequent Injection procedure

the WHO 2010 (World Health Organization, 2010) were used to classify the semen samples according to quality.

2.3 | Sperm selection procedure

2.3.1 | Density gradient centrifugation

For density gradient (DG) processing, the sperm sample was gently layered above the two-layer gradient (2 ml 45%, 2 ml 90%) (Spermient 100%, Cook Medical) and centrifuged for 20 min at 400 *g*. Thereafter, the supernatants containing the seminal plasma were discarded and the pellet was washed with 6 ml of gamete buffer (Cook Medical) for 10 min centrifugation at 400 *g*. Again, the supernatant was discarded until 0.5 ml above the pellet in which the pellet was resuspended. Twenty-five microlitres of this suspension was used for SDF testing, and the remaining part was further processed for either Swim-out, Swim-ICSI or MACS®.

2.3.2 | Recovery of spermatozoa with progressive motility by Swim-out and Swim-ICSI

Both in-house swim-out methods were designed to recover and select progressive motile (A + B) spermatozoa based on the trajectory that spermatozoa follow in HEPES-buffered medium. Detailed methodological design is shown in Figure 2.

Conventional Swim-out

A rectangle of 0.4 ml incubated HTF HEPES 0.4% HSA (Gynotec) (3.5 cm on 2 cm) with 4 foothills (0.1 ml HTF HEPES 0.4% HSA) was made in a 60-mm Petri dish (Falcon®) and covered with 8 ml of incubated light mineral oil (Irvine Scientific). After preparing the swim-out dish, 11.5 µl of the sperm fraction after DG was added to the right lower corner of the swim-out and progressive spermatozoa were migrating through the HEPES-buffered medium towards the foothills according to their motility (Figure 2a). The swim-out was incubated for approximately 2 hr on RT, and thereafter, progressive motile spermatozoa were recovered manually in the top of the furthest foothill. In case of ICSI treatment, these motile spermatozoa would be added to the sperm droplet in a new ICSI dish (Vitrolife) as shown in Figure 2a but for this experiment they were collected in a small tube with HTF HEPES 0.4% HSA for further SDF testing.

Swim-ICSI

As shown in Figure 2b, for the Swim-ICSI, an ICSI dish (Vitrolife) was prepared with six droplets of 0.8 µl HTF HEPES 0.4% and covered with 4 ml of incubated light mineral oil (Irvine Scientific). The upper middle droplet was used for the recovery of the progressive motile spermatozoa (A + B), and the other droplets could be used for the oocytes during the ICSI procedure. The upper middle droplet was connected through a small bridge of HTF HEPES 0.4% HSA with a small HTF HEPES 0.4% HSA square of approximately 1 by 4 cm with HEPES where 11.5 µl of the sperm fraction after DG was added in each corner. Subsequently, the ICSI dish was incubated at RT for the migration of the progressive (A + B) motile to the sperm droplet. Time of incubation for the recovery of the motile spermatozoa was depending on the motility of the semen sample and was about 20 min on average. When sufficient spermatozoa were present in the sperm droplet, the bridge was disconnected with a sterile tip, so that spermatozoa could not migrate into the sperm droplet anymore. In case of ICSI treatment, this sperm preparation dish can be used for ICSI after incubation on 37°C. In this experiment, the recovered spermatozoa in the sperm droplet were collected for SDF testing.

2.3.3 | Isolation of non-apoptotic spermatozoa by Magnetic cell sorting (MACS®)

The commercial MACS® ART Annexin V System (Miltenyi Biotec, 2016) distinguishes apoptotic sperm cells with the help of the molecule Annexin V (ANV). This molecule has a high affinity for phosphatidylserine at the outer leaflet of the plasma membrane of spermatozoa with activated apoptosis signalling or membrane damage (Grunewald & Paasch, 2013) and is magnetically labelled with microbeads. 150 µl of the washed sperm fraction after DG was mixed with the magnetically labelled ANV molecules (100 µl of Annexin V Reagent and 400 µl of Binding Buffer) and was incubated for 15 min at room temperature (RT). The ANV molecules bind with phosphatidylserine on the apoptotic spermatozoa, labelling the apoptotic sperm cells magnetically. This cell suspension was loaded on a separation column which was placed in a magnetic field. The magnetic beads (= the apoptotic spermatozoa) were attracted with the magnet while the remaining unbound ANV- (MACS® -) spermatozoa without apoptosis could elute and were collected in a tube for further SDF testing.

2.3.4 | Sperm DNA fragmentation testing

Sperm DNA fragmentation was assessed using the Halosperm® test (Halotech). This commercial invasive diagnostic kit is an indirect and valid SDF test that allows the measurement of SDF in an easy, fast and reproducible manner without the requirement for expensive laboratory equipment. This method is based on the Sperm Chromatin Dispersion technique which is based on controlled DNA denaturation to facilitate the subsequent removal of the nuclear proteins contained in each spermatozoon. In this way, normal spermatozoa create halos formed by loops of DNA at the head of the spermatozoa, which are not present in those with damaged DNA.

The sperm fractions were diluted with gamete buffer (Cook Medical) to obtain a concentration of 5–10 million sperm cells/ml. Then, 25 µl of spermatozoa was mixed in the tube with agarose gel, which had previously been warmed in a Thermo-shaker (Biosan) for 5 min on 100°C and 5 min on 37°C. Next, the semen-agarose solution was placed onto a pre-coated slide and covered with a coverslip.

The slides were refrigerated at 4°C and left for 5 min to solidify the agarose. Subsequently, the coverslip was gently removed and the pre-coated slide with solidified agarose-sperm solution was incubated for 7 min in the denaturation acid (80 µl in 10 ml aqua purificata) at RT. Immediately afterwards, the slide was incubated in 10 ml lysis solution at RT for 20 min to remove most nuclear proteins. The lysis solution was then washed off with aqua purificata (two times a 5 min bath at RT). After washing, the slides were fixed by incubating them in 70% (2 min, RT), 90% (2 min, RT) and 100% (2 min, RT) ethanol. The slides were then left to dry at RT. In the final step, the slides were stained with the Wrights staining (7-min red eosin solution, 7-min blue Azure B solution, 1-s rinsing in aqua purificata; RT) and left to dry for brightfield microscope observation using a 40× objective. Spermatozoa without DNA fragmentation showed a typical halo of scattered DNA loops. Spermatozoa with large- or medium-sized halos were classified as DNA intact, whereas spermatozoa with small, no or degraded halos were classified as DNA fragmented spermatozoa. Only spermatozoa with tail were included in the analysis.

To determine the SDF rate, 200 spermatozoa were counted by two different assessors for each sample. The SDF rate was calculated as the number of counted spermatozoa with SDF divided by the number of spermatozoa counted multiplied by 100. An average of the SDF rate of 2 assessors was taken as the average SDF rate.

2.4 | Statistical analysis

Statistical analysis was performed using SPSS version 26 (Germany). Comparisons of the SDF between the native semen, after DG, after conventional Swim-out and Swim-ICSI were carried out using the Friedman test. For pairwise comparisons of two methods, the Wilcoxon signed-rank test was used. A $p \leq .05$ was considered statistically significant.

3 | RESULTS

3.1 | Basic semen characteristics of male patients

Fresh semen ejaculates of 42 ICSI patients (mean age of 38 ± 6.4 years) showed a mean volume of 3.31 ± 1.5 ml, a mean concentration of 83.48 ± 0.99 million per ml and a mean progressive motility (A + B motility) of 46.34 ± 20.14%. According to the WHO 2010 criteria (World Health Organization, 2010), 30 samples (71.4%) were classified as normospermia, 1 (2.4%) as oligospermia, 7 (16.7%) as asthenospermia and 4 (9.5%) as oligoasthenospermia. The basic semen characteristics are described in Table 1.

3.2 | Sperm DNA fragmentation

A total of 168 SDF tests were performed. In the first analysis, the SDF rate of all 42 semen samples was assessed and compared for the native sample, after DG, after DG + conventional swim-out and after DG + Swim-ICSI. The results of this comparison are presented in Figure 3. The mean SDF rate was significantly lower in the sperm pellet after DG compared to the native semen sample, 13.78 ± 12.39% versus 4.58 ± 5.65%, respectively ($p < .001$). The mean SDF of the recovered motile (A + B) spermatozoa was reduced to almost zero when the prepared semen sample after DG was further processed for ICSI using the conventional swim-out method or the more recent designed swim-ICSI. The mean SDF rate was 0.42 ± 1.14% post-DG + swim-ICSI and significantly lower than post-DG + swim-out where the mean SDF rate was 0.89 ± 1.84% ($p = .001$).

In a sub-analysis of 20 semen samples, both in-house designed ICSI preparation methods were compared with the commercial MACS® ART Annexin V test. The mean SDF rate was significantly lower after the selection of motile (A + B) spermatozoa post-DG + swim-ICSI compared with the selection after DG + swim-OUT and of the Annexin V-negative spermatozoa selected with MACS®; 0.43 ± 1.51%[a,b], 1.03 ± 2.47%[a] and 1.69 ± 3.57%[b], respectively ($p \leq$ [(a)]0.003, [(b)]0.001) (Figures 3 and 4).

TABLE 1 Basic semen characteristics

Parameter	Value
Number of patients	42
Mean male age (years)	38 ± 6.4
Mean volume (ml)	3.31 ± 1.5
Mean sperm concentration (million/ml)	83.48 ± 0.99
Mean progressive sperm motility (%)	46.34 ± 20.14
Semen analysis	
Normospermia	30 (71.4%)
Oligospermia	1 (2.4%)
Asthenospermia	7 (16.7%)
Oligoasthenospermia	4 (9.5%)

Note: Values are expressed as mean ± *SD*; progressive motility is expressed as A + B motility.

4 | DISCUSSION

Several methods are available and widely used for semen preparation for ICSI, but there is no consensus on which method is more suitable than another. Each of these methods, based on the migration or separation of spermatozoa, is useful in selecting spermatozoa with suitable morphology and motility but are not directly capable in selecting spermatozoa without sperm DNA damage (Volpes et al., 2016). All current methods to determine SDF are diagnostic and invasive, so that spermatozoa can no longer be used for treatment. It is important to be able to select spermatozoa without DNA damage (Oseguera-López et al., 2019). Despite the lack of clarity regarding associations between SDF and conventional semen parameters, there are some studies that have shown relationships between semen parameters and sperm DNA damage (Frydman et al., 2008;

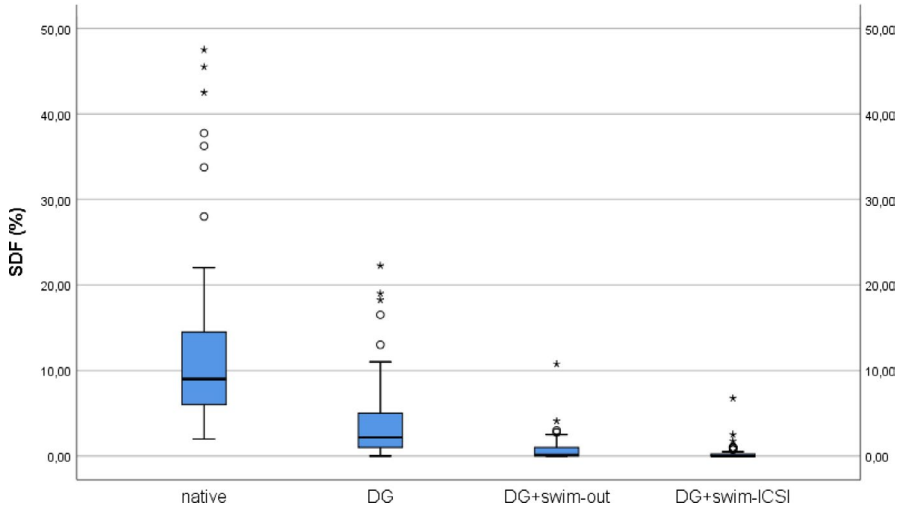

FIGURE 3 Relative reduction in sperm DNA fragmentation rate after sperm preparation methods density gradient (DG), DG + swim-out, DG + swim-ICSI compared to the native sample (for all 42 semen samples). Statistical significance $p < .05$, Friedman test and two-by-two comparison with the Wilcoxon signed-rank test: ≤0.001 for all 2 on 2 comparisons. Box plots: the bottom and top of the box represents the first and the third quartile, the band inside the box refers to the median, the whiskers indicating the variability outside the upper and lower quartiles (a distance of 1.5 times the inner quartile range). °= mild outliners, *= extreme outliners

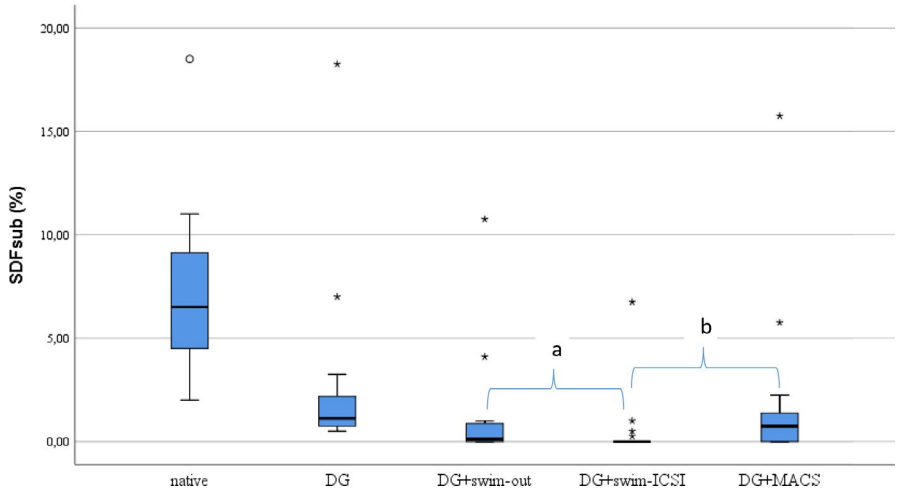

FIGURE 4 Sperm DNA fragmentation rate after sperm preparation methods density gradient (DG), DG + swim-out, DG + swim-ICSI and DG + MACS compared to the native sample in a sub-analysis of 20 semen samples. Friedman test and two-by-two comparison with the Wilcoxon signed-rank test showed significant differences between DG + swim-out and DG + swim-ICSI ($p = .003$) (a) and between DG + swim-ICSI and DG + MACS ($p = .001$) (b) Box plots: the bottom and top of the box represents the first and the third quartile, the band inside the box refers to the median, the whiskers indicating the variability outside the upper and lower quartiles (a distance of 1.5 times the inner quartile range). °= mild outliners, *= extreme outliners

Greco et al., 2005; Larson-Cook et al., 2003; Tomlinson et al., 2001; Virro et al., 2004). Simon et al. (Simon & Lewis, 2011) and Palermo et al. (2014) observed a significant negative correlation between sperm DNA damage and only one conventional parameter that of sperm motility.

In our centre, we have been using a specific semen swim-out preparation method for selecting spermatozoa for ICSI for many years. This procedure is based on a swim-up principle with the difference that the spermatozoa actually do not swim-up, but they swim according alongside the medium barrier of a defined structure. We hence call it a swim-out instead of a swim-up. Previous in-house validations had shown that this sperm preparation swim-out method was capable of selecting highly motile spermatozoa that had a low SDF (unpublished data). Our conventional swim-out method comprised of a two-step ICSI preparation step where the swim-out selected spermatozoa had to be transferred from the swim-out dish to the ICSI dish. In order to optimise this procedure to an all-in-one step procedure, another swim-out configuration was designed. This new procedure, called Swim-ICSI, comprised of the selection and the ICSI procedure in 1 dish, eliminating the dish transfer (a one-step approach). Because the swim-out time

and the distance of the sperm's swimming were extensively reduced, it could be that spermatozoa with higher DNA fragmentation could end up in the final collection droplet. Therefore, a validation experiment was set up combining the former methodology with the new concerning SDF of the collected spermatozoa. Additionally, the MACS methodology was added to the experimental setting to verify how our in-house protocols could match the results of this commercial kit.

Our results showed that the mean SDF rate was significantly lower in the sperm pellet after density gradient compared to the native semen sample, 13.78 ± 12.39% versus 4.58 ± 5.65%, respectively ($p < .001$). The SDF in the final collected semen sample after density gradient and swim-out was further reduced to 0.89 ± 1.84%. The new swim-ICSI method, although a lot faster in execution, was able to significantly reduce the SDF even further to 0.42 ± 1.14% ($p = .001$). These results show that density gradient centrifugation followed by a swim-out method is very effective for the selection of spermatozoa with minimal sperm DNA damage. In addition to the capability of selecting spermatozoa with very low up to undetectable DNA fragmentation, the new designed swim-ICSI is also less labour intensive, cheaper and requires fewer identification steps which makes it a very effective and safe method for selecting spermatozoa for ICSI.

In a sub-analysis, the in-house designed swim-out methods were compared to the commercial Magnetic-activated cell sorting test (MACS® ART Annexin V, Miltenyi Biotec, 2016). Several studies have shown the potential of MACS selected spermatozoa for fertility treatment (Martínez et al., 2018; Sánchez-Martín et al., 2017). These studies assumed an association of sperm apoptosis with sperm DNA damage (Martínez et al., 2018; Sánchez-Martín et al., 2017), and therefore, SDF is often used as outcome parameter in studies comparing semen preparation methods with MACS®. The mean SDF rate was significantly reduced after density gradient and MACS® (1.69 ± 3.57%) compared with density gradient prepared spermatozoa (2.46 ± 7.70%) or native spermatozoa (7.01 ± 4.31%) ($p \leq .001$). However, our results show that the combination of density gradient and MACS was not superior to density gradient in combination with a swim-out technique. Although already very low, the SDF was still significantly higher with MACS® compared with conventional swim-out and the new swim-ICSI, 1.69 ± 3.57^{ǂ} and 1.03 ± 2.47$^{*\ ǂ}$ and 0.43 ± 1.51$^{*\ ǂ}$, respectively ($p \leq$ *.003, ^{ǂ}.001).

Our results after MACS are in line with previous reports (Grunewald et al., 2006; Zahedi et al., 2013; Lee, Liu, & Lee, 2010). In each case, a significant drop sperm DNA damage is seen after determining the mean SDF of the native sample, after DGC and after MACS (combined with DGC) and suggest that MACS or DCG combined with MACS seem more effective for the selection of SDF-free spermatozoa than DGC alone and this is in accordance with the results of the sub-analysis in our report. An interesting point to mention which may have had an impact on the results of SDF-free sperm selection after DGC + MACS is that the selection of spermatozoa after MACS is based on the externalisation of phosphatidylserine (EPS). Externalisation of phosphatidylserine (the phospholipid that binds to the molecule Annexin V during the MACS analysis) is considered as an early sign for apoptosis (Martin et al., 1995). This process, however, can also occur following capacitation and acrosome reaction. The separation of spermatozoa from the seminal fluid during DGC using media containing albumin, as is the case in our procedure, can facilitate capacitation as well (Grunewald et al., 2006; Salicioni et al., 2007). Therefore, it is not clear whether Annexin-positive spermatozoa removed from the sample post-DGC are residues of apoptosis and DNA damage before ejaculation, or EPS after capacitation and acrosome reaction due to the presence of albumin in our density gradient medium. In other words, there is a possibility of de-selecting hyperactivated spermatozoa having undergone capacitation, because the gradient density centrifugation was performed before the MACS. The study of Tavalaee et al. (2012) investigated this aspect and demonstrated a lower, not significant, amount of DNA fragmentation after MACS-DGC than when the order of the techniques is reversed as is the case in this study: DGC-MACS.

Although this study shows a reduction in SDF in the selected sperm populations, there are some limitations to the set-up. Recovery rates after the density gradient centrifugations were not analysed, since it is not a standard procedure to do this in our centre. A specific volume of the final fraction containing motile spermatozoa was used for subsequent preparation techniques in this study. This means that only a rough estimate of the sperm concentration used as input in the subsequent ICSI swim-out techniques was known. The sample size, although higher than in similar studies in literature, is still quite small. Moreover, almost all samples had normal SDF levels in the native samples (13.78 ± 12.39%). Thirty-six of the 42 samples had an SDF index below the cut-off of 30% which may influence the decrease to almost zero after sperm preparation and 30 of the examined samples were normospermic. Nevertheless, the difference between the conventional swim-out and the new swim-ICSI remains significant. Additionally, the sole outcome parameter of this study is SDF.

In conclusion, standard semen preparation techniques like density gradient centrifugation clearly result in a semen fraction with lower sperm DNA damage. However, when spermatozoon is further processed by subsequent preparation techniques, SDF almost declines to zero. The swim-ICSI preparation method, where semen is allowed to swim along a medium line and can be collected in a droplet, shows the possibility to select a population of spermatozoa with almost 0% SDF. This all-in-one preparation and ICSI dish make it possible to select highly motile, morphologically normal spermatozoa with low SDF for ICSI in an easy and straightforward way.

ACKNOWLEDGEMENTS

We kindly acknowledge all colleagues from the Andrology and IVF lab who helped with the practical work for the experiments. We are grateful for statistical guidance by Roos Colman of the Biostatistics Unit of Ghent University.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (Stefanie De Gheselle), upon reasonable request.

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How to cite this article: De Gheselle S, Deroose A, Stevens J, Hiel M, Tilleman K. A methodological validation of an easy one-step swimout semen preparation procedure for selecting DNA fragmentation-free spermatozoa for ICSI. *Andrologia*. 2020;52:e13852. https://doi.org/10.1111/and.13852