Direct swim-up without centrifugation is a more recommended technique for sperm preparation in conventional IVF cycles

Fang-Ting Lu
The First Affiliated Hospital of University of Science and Technology of China
Ren-Tao Jin
Reproductive and Genetic Hospital, The First Affiliated Hospital of USTC
Bo Xu
The First affiliated Hospital of USTC
Li-Min Wu
The First affiliated Hospital of USTC
Na-Ru Zhou
The First affiliated Hospital of USTC
Jing-Yao Li
The First Affiliated Hospital of USTC
Hong-Bing Luan
The First Affiliated Hospotal of USTC
Yu-Sheng Liu
The first affiliated Hospital of USTC
Xian-Hong Tong (tong68xianhong@163.com)
Reproductive and Genetic, The First Affilated Hospital of University of Science and Technology of China
https://orcid.org/0000-0003-2894-9085

Research

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Abstract

Backgroud: The aim of this study is to investigate the most effective and maneuverable technique for sperm preparation in conventional IVF cycles.

Method: A retrospective and laboratory-based study was conducted in patients who underwent their first cycle of IVF from January to December in 2016 to compare two sperm preparation techniques: direct swim-up without centrifugation (DSU) technique and density-gradient centrifugation followed by swim-up (DGC-SU) technique. A series of experiments in this study was designed to evaluate the efficiency of these two techniques which include: (i) assessment of quality and quantity of spermatozoa by comparing motility, DNA fragmentation index (DFI), acrosomal reaction (AR) and mitochondrial membrane potential (MMP) of DSU-separated sperm to DGC-SU-separated sperm, (ii) evaluation of safety of DSU technique by assessing the risks of bacterial contamination, (iii) analysis of feasibility of replacing DGC-SU with DSU technique by reviewing ART outcomes including fertilization rate, high-quality embryo rate, implantation rate, clinical pregnancy rate, take-home baby rate and abortion rate.

Results: Although there were no significant differences in DFI, AR and MMP between DSU-separated sperm and DGC-SU-separated sperm, significantly higher percentage of progressive motility in DSU-separated sperm were found than that in DGC-SU-separated sperm. Moreover, there were no significant differences between DSU and DGC-SU groups on ART outcomes based on data of fertilization rate, high-quality embryo rate, implantation rate, clinical pregnancy rate, baby delivery rate and abortion rate. In addition, no bacterial contaminations were found in culture medium samples of simulating fertilization from DSU group. However, it is noticeable that DSU technique required less time and labor for sperm preparation compared with DGC-SU.

Background

With the development of assisted reproductive technology (ART), it is widely accepted that high-quality sperms are required to produce good-quality embryos which is a major determinant of ART success [1–3]. Because of that, multiple techniques for sperm preparation have been developed for IVF and ICSI treatments.

Swim-up and density-gradient centrifugation (DGC) are the two main techniques which are used to separate the functional motile spermatozoa from the other semen components in ART cycles. These two techniques are based on different underlying principles. Swim-up is designed to isolate highly motile spermatozoa and DGC is designed to separate spermatozoa depending on chromosome packaging. Both of them are useful in selecting suitable morphology and motility sperm cells. However, several studies reported that a combination technique of density-gradient centrifugation and swim-up (DGC-SU) was more effective in enriching sperm cells with normal morphology and eliminating sperm cells with DNA fragmentation than DGC technique alone [4–7]. Given this, the DGC-SU technique is more widely used in the field of ART work concerning sperm preparation compared to DGC technique alone at the present time in China.

However, DGC-SU is a tedious procedure, which requires excessive manipulation and multiple centrifugations. Several studies have shown that excessive manipulation and multiple centrifugations can lead to an increase of sperm DNA fragmentation mediated through production of reactive oxygen species (ROS) by spermatozoa, cellular debris and leucocytes [8–11]. Also, recent studies have reported that DGC did not decrease sperm DNA fragmentation as previously reported [12–13]. High level of sperm DNA fragmentation is associated with a low chance of both natural and artificial conception [14–18]. More importantly, in a study by Aitken et al (2014), it revealed that colloidal silicon-based discontinuous density gradients that are commonly used to prepare human spermatozoa for assisted reproduction technology (ART) purposes contain metals that promote free radical-mediated DNA damage. This finding suggests that density-gradient centrifugation should be avoided during sperm preparation [19]. In addition, the DGC-SU technique requires operators to transfer sperm sample among several tubes, thereby increasing the potential risk of mismatches [5, 20–21]. These limitations, in concert, suggested that the DGC-SU was not optimal technique for sperm preparation for IVF and ICSI treatments.

The DSU is a simple, fast and economical technique for sperm preparation without centrifugation. It has been reported that a micro direct swim-up without centrifugation (MSU) in ICSI treatment was a better choice for sperm preparation than the DGC-SU procedure [20–21]. However, there are no studies on whether this technique is a favorable choice for sperm preparation in conventional IVF treatment.

In the study reported herein, we primarily aimed to evaluate the effect of sperm preparation with DSU or DGC-SU technique on quality and quantity of recovering spermatozoa based on recovery rates of progressive motile sperm, levels of sperm DNA fragmentation and sperm AR and MMP. In addition, we further assessed the DSU technique for sperm preparation by reviewing ART outcomes of IVF cycles between DSU and DGC-SU groups including fertilization, embryo development and pregnancy.

Methods

Patients

A retrospective study was conducted in patients who underwent their IVF cycles in Reproductive and Genetic Hospital of the First Affiliated Hospital of University of Science and Technology of China (Hefei, Anhui, China), from January to December 2016. A total of 1223 IVF couples were included in this comparison: 536 couples prepared sperm with DSU technique, 687 couples with DGC-SU technique. The inclusion criteria for the female partner were: (i) age <40 years; (ii) body mass index (BMI) <30 kg/m^2; (iii) basal level of follicle-stimulating hormone (FSH) <10 IU/L; and (iv) undergoing their first cycle of IVF. The female partners with diagnosis of uterine or karyotype abnormality were excluded. The study was approved by Ethics Committees on Human Research of the First Affiliated Hospital of University of Science and Technology of China. There is no conflict of interest in the present study.

Preparation of semen samples

Semen samples were obtained by masturbation after at least a 2-day sexual abstinence. After liquefaction at room temperature for 20 minutes, raw semen samples were examined for concentration and motility according to the 2010 WHO guidelines. Spermatozoa were categorized by the following different motility grades: 1) progressively motile, 2) non-progressively motile, and 3) immotile sperm. In order to reduce the artificial differences in sperm characteristics, the routine semen analyses were performed by the same well-trained technician using Computer-aided sperm analysis (CASA). A 500μl semen sample was drawn to evaluate the basal level of sperm motility, viability, DNA fragmentation, high DNA stainable (HDS), acrosomal reaction (AS), mitochondrial membrane potential (MMP), and the remaining sample was split into two aliquots for sperm selection. One aliquot was separated with the DSU technique, and another aliquot was separated with the DGC-SU technique. Similarly, DSU-separated sperm and DGC-SU-separated sperm were examined for motility, viability, DNA fragmentation, HDS, AS, MMP and microbiological contamination.

Density-gradient centrifugation followed by swim-up (DGC-SU)

1ml of semen sample was layered on the top of the discontinuous density gradients (40 and 80%, ORIGIO, Denmark) and centrifuged for 20 min at 350g. Thereafter, sperm pellet at the bottom of tube was collected. At least 3 ml of the culture sperm medium (IVF Sperm Medium, COOK, Limerick) was added to washing sperm pellet and the tube was centrifuged for 6 min at 250g. The supernatant was discarded and 2 ml of the same culture sperm medium was layered gently above the pellet. The tube was inclined at an angle of 45 degrees and incubated for 40 min at 37°C with 6% CO_2, and then 1 ml of the middle-clouded layer medium was collected into a new tube for the following experiments [22]. The whole process of DGC-SU takes around 75 minutes (Fig. 1).

Direct swim-up without centrifugation (DSU)

2 ml of the culture sperm medium was prepared into a round-bottomed tube, and then 1ml of semen sample was added to the bottom of the tube. The tube was inclined at an angle of 45 degrees and incubated for 40 min at 37°C with 6% CO_2, and then 1 ml of the middle-clouded layer medium was collected into a new tube for the following experiments. The whole process of DSU only takes about 45 minutes (Fig. 1).

Measurement of sperm DNA damage

The level of sperm DNA damage was assessed by DNA fragmentation index (DFI) as described previously [23–24]. Briefly, about one million spermatozoa were treated with hydrochloric acid on ice for 30 sec and then incubated for about 1 min at room temperature with acridine orange (AO, Cellpro Biotech, Zhejiang, China) solution. The spermatozoa were then analyzed by flow cytometry (Accuri C6 cytometer, BD Biosciences, San Jose, CA). More than 5,000 spermatozoa were collected per sample in the gate of sperm population. Data was analyzed using the flow cytometer software (Cellpro Biotech). AO intercalated with

double-stranded DNA emits green fluorescence and AO associated with single-stranded DNA emits red fluorescence. The DFI value was calculated by the ratio of red to total (red plus green) fluorescence intensity. Additionally, the fraction of HDS sperm cells was also detected in this experiment. HDS represents immature spermatozoa in semen with incomplete chromatin condensation, which exhibit green fluorescence intensity higher than the upper border of the main cluster of the sperm population.

Evaluation of the acrosomal reaction

Acrosomal reaction (AR) was evaluated by FCM according to manufacturer's instructions (Cellpro Biotech). In Brief, sperm samples were first diluted and swam up in culture medium for 15 min. After 2-minute fixation in 75% ice ethyl alcohol, calcium ion carrier A23187 (Sigma-Aldrich, St Louis, MO) was added to the sperm suspension to make a final concentration of 10 μmol/L A23187. A23187 stock solution (5mmol/ L) in dimethyl sulfoxide (DMSO, Sigma-Aldrich) was frozen at −20°C. Before use, this was thawed, dissolved in human tubal fluid (HTF) medium. As a control, diluted DMSO (Sigma-Aldrich) was added to the same sperm suspension. Both test and control tubes were incubated for 15 min at 37°C with 5 μg/ml of FITC-conjugated pisum sativum agglutinin (FITC-PSA, Cellpro Biotech) working solution in the dark. Next, spermatozoa were analyzed by FCM. At least 5,000 events were collected per sample in the gate of sperm population. Acquired data was analyzed by flow cytometer software. When more than half the head of a spermatozoon was fluoresced brightly and uniformly with FITC-PSA, the acrosome was considered to be intact. Spermatozoa without fluorescence or with a fluorescing band limited to the equatorial segment were considered to be acrosome-reacted. The ionophore-induced AR was calculated by the acrosome-reacted ratio of test group subtract that of control group.

Measurement of MMP

The mitochondrial membrane potential (MMP) was examined according to the manufacturer's instructions (Cellpro Biotech). Briefly, one million spermatozoa were incubated with 2 μM of the lipophilic cationic dye 5,5',6,6'-tetrachloro−1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine iodide (JC−1) working solution for 15 min at 37°C in the dark. Afterwards, the sperm cells were analyzed by FCM. More than 5,000 spermatozoa were collected per sample in the gate of sperm population. JC−1 exists in the cell in the form of polymers and monomers. When the MMP is high, JC−1 spontaneously forms polymers with red fluorescence. When

the MMP is low, JC–1 is mainly in the form of monomers and exhibit green fluorescence. The value of MMP was calculated by the ratio of red to total fluorescence using the flow cytometer software.

Microbiological examination

Native semen samples were collected in sterile plastic containers by masturbation in the IVF laboratory. After liquefaction, the semen sample was split into two aliquots for sperm selection. One aliquot was separated with the DSU technique, and another aliquot was separated with the DGC-SU technique. All reserved specimens, including native semen, the upper layer medium after DGC-SU and DSU treatments and the culture medium of simulating fertilization test were collected in sterile plastic containers and examined for aerobic and facultative bacteria within 30 minutes of collection. 10μl sample was inoculated on blood and chocolate agar plates respectively, followed by incubation for 48 hours in 5% CO_2 at 37°C. Next, the presence, pattern and number of colonizing bacteria were checked. Identification of bacteria was performed in that case either by means of the Vitek^{TM} system (bioMerieux, St. Louis, MO) or a conventional method, as required. All operations of microbiological examinations were carried out in the biosafety cabinets.

IVF protocols

IVF was carried out following routine protocols [25–26]. Briefly, ovarian stimulation of multiple follicles was achieved by purified (FSH, LiZhu pharma, ZhuHai, China) or recombinant (Gonal-f, Merk Serono SA, Geneva, Switzerland) gonadotropins. At 36 h after administration of 10, 000 IU human chorionic gonadotropin (hCG, LiZhu pharma), cumulus-corona oocyte complexes (COCS) were retrieved by vaginal ultrasound-guided aspiration.

Native semen samples were collected in sterile plastic containers by masturbation in the IVF laboratory. All patients were asked to wash their hands and genital areas with soap and water before sperm collection. After liquefaction, semen samples were randomly divided into two groups: one group selected sperm for IVF with DSU technique, and another group selected sperm for IVF with DGC-SU technique. Sperm selected by DSU or DGC-SU technique were used for subsequent insemination of the oocytes at a final concentration of 200, 000 sperm/ml. After co-incubation with spermatozoa for 4 h, the oocytes were completely denuded by pipetting and checked for the presence of the second polar body. The early rescue ICSI were performed in patients who had more than 2/3 oocytes failed to extrude the second polar body after 6 h of insemination [27]. 16 to 18 h after insemination, oocytes were assessed for 2 pro-nuclei presence. 72 h after oocyte retrieval, embryos were classified according to their morphology. Up to two day 3 embryos with good quality were transferred into uterus.

Pregnancy outcomes

A biochemical pregnancy was diagnosed only by the detection of beta hCG in serum or urine 14 days after embryo transfer. Ultrasound was performed at 6 weeks after embryo transfer to confirm clinical pregnancy. A clinical pregnancy was diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. In addition to intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy. Spontaneous abortion was defined as a pregnancy loss of an intra-uterine pregnancy prior to the 22th week of gestation [28]. The implantation rate was calculated as the number of gestational sacs divided by the total number of transferred embryos.

Statistical Analysis

The statistical analysis was performed by using the SPSS, version 20 software. One-way ANOVA was performed to compare the differences among the three groups and the data shown was described as mean ± standard deviation (S.D). Differences regarding rates were evaluated by the Chi-Squared test and the data shown was described as percent (%). *p*-values <0.05 were considered statistically significant.

Results

Recovery rate

As noted in Table 1, although both DSU and DGC-SU techniques were able to significantly increase proportions of sperm with progressive motility compared to unprocessed native semen, DSU technique improved significantly higher percentage of progressive motility compared with DGC-SU technique (Tab.1). However, there was no significant difference on the recovery rate of progressive motile sperm after between DSU and DGC-SU (Fig. 2).

Sperm DNA fragmentation and other indicators of sperm quality

Data concerning the assessment of sperm DNA integrity was summarized in Table 1. Sperm DNA integrity was expressed as DNA fragmentation index (DFI) and high DNA stainable (HDS) cell proportion. Compared with native semen, both DSU and DGC-SU techniques were able to decrease the DFI of sperm. There was no significant difference on the DFI of sperm between DSU-separated and DGC-SU-separated sperm (Tab.1). Similarly, both DSU and DGC-SU techniques led to significant reduction of HDS sperm compared with native semen, but no significant difference was found between these two groups (Tab.1).

Moreover, as it was shown in Table 1, both DSU and DGC-SU techniques could significantly increase levels of sperm AR and MMP compared with native semen. However, DSU technique did not increase levels of sperm AR and MMP relative to DGC-SU technique (Tab.1). Taken together, these results indicate that both DSU and DGC-SU techniques are able to recover high-quality sperm.

Safety concern

As it was shown in Supplementary table 1, bacterial contaminations were found in 64 of 92 native semen samples. Gram-positive cocci such as *streptococcus, staphylococcus and enterococcus* were the most common bacterial contaminants. The same bacterial examinations were repeated after sperm preparation. Only one upper layer medium sample showed *streptococcus* contamination after DSU treatment, while no bacterial contamination was found in 88 upper layer medium samples after DGC-SU treatment (Supplementary tab.1). Moreover, there was no bacterial contamination in the culture medium of simulating fertilization test from both the DSU and DGC-SU group (Supplementary tab.1). Taken together, these results suggest that neither DSU nor DGC-SU technique causes bacterial contamination.

Laboratory and clinic outcomes in IVF cycles

A total of 1223 IVF couples were included in this comparison: 536 couples prepared sperm with DSU technique, 687 couples with DGC-SU technique (Fig. 3). Demographic data were given in Table 2. No significant differences between the DSU and DGC-SU group were observed in the female partner's age, BMI, infertility duration, indication of IVF and basal levels of sex hormones (Tab. 2).

As it was shown in Table 3, there were no significant differences on the laboratory outcomes of IVF treatment involving fertilization rate, rescue ICSI rate, cleavage rate and high-quality embryo rate between the DSU and DGC-SU group. In addition, clinic outcomes of IVF treatment were summarized in Table 4. 227 of 536 patients in the DSU group and 310 of 687 in the DGC-SU group underwent fresh-embryo transfer. The average number of embryos per transfer was similar in these two groups (Tab. 4). No significant difference between the DSU and DGC-SU group was observed on the clinic outcomes of IVF cycles, with regard to chemical pregnancy rate, clinical pregnancy rate, implantation rate, and take-home baby rate (Tab. 4). Moreover, spontaneous abortion rate also showed no difference between the DSU and DGC-SU group (Tab. 4). Therefore, we concluded that both DSU and DGC-SU are effective techniques for sperm preparation in terms of fertilization, embryo development and pregnancy in IVF cycles.

Discussion

Elimination of damaged spermatozoa and selection of the best-quality spermatozoa are essential for successful IVF cycles. At the present time, the most prevalent technique for sperm preparation is a combination of density-gradient centrifugation and swim-up (DGC-SU) in China [22, 29]. For this combination technique, swim-up procedure was performed after the elimination of harmful components by density-gradient centrifugation. DGC-SU has been reported to be favorable for sperm preparation in IVF cycles, as spermatozoa prepared by this technique were found to have higher rates of motility and reduced DNA fragmentation compared with other procedures [4–5, 22].

However, A study by Aitken and colleagues has shown that the use of discontinuous density gradients for sperm preparation significantly contributed to elevated oxidative DNA damage. In their study, it was revealed that this medium contains metals, particularly Fe, Al and Cu, which are known to promote free radical generation in the immediate vicinity of DNA. Moreover, metal contamination is a relatively constant

feature of such products [19]. This fact suggests that density-gradient centrifugation should be avoided during sperm preparation. Thus, we investigated a simple, fast technique for sperm preparation in IVF cycles, namely direct swim-up without centrifugation (DSU). Compared with the conventional pellet swim-up technique, the DSU technique does not require single or multiple centrifugation steps. It has been widely accepted that the centrifugation step damages spermatozoa via the generation of ROS [30–32]. In addition, the DSU technique isolated high-quality spermatozoa directly from seminal plasma. Seminal plasma has powerful antioxidant capacity that can protect spermatozoa from ROS. There was a significant increase in the levels of ROS generated by samples which were prepared by swim-up from a washed pellet compared with samples isolated directly from seminal plasma [32]. Elimination of seminal plasma can lead to oxidative attack on sperm DNA [33]. These findings suggest that the DSU technique may be recommended to use for sperm preparation in IVF cycles.

In this study, firstly, we evaluated the quantity and quality of recovering spermatozoa after DSU treatment. It was demonstrated that DSU technique was able to recover enough progressive spermatozoa. A comparison between DSU and DGC-SU showed that sperm separated with DSU technique had higher percentage of progressive motility than that with DGC-SU technique. This is contrary to the finding that there were no significant differences between these two sperm preparation techniques [22]. The possible reason of different findings was due to different sperm preparation time and different gradient densities. Sperm DNA fragmentation is negatively correlated with ART outcomes and was identified as a major indicator of sperm quality [17–18, 24, 31, 34]. AR and mitochondrial biomarker MMP also can be regarded as predictors of semen quality in the general study population [35–39]. No difference was observed on the level of DFI, MMP and AR between these two techniques. Therefore, we concluded that DSU technique could select high-quality sperm similar with DGC-SU technique. This result is in agreement with the findings of Li et al. (2012) who found no significant difference between these two techniques in terms of the sperm changes in MMP and ROS [22]. However, our results were inconsistent with the findings presented by Ghaleno and colleagues [40]. They found that the separated sperm with DSU technique were not better than that with DGC-SU technique on the sperm motility and level of MMP. We speculated that incubations at room temperature (RT) but not 37°C in their study result in these differences. Some study reported that 37°C incubation resulted in higher motility relative to RT incubations [41–42]. In addition, our results indicated that the level of sperm DFI after processing by DSU is similar with that by DGC-SU, which was detected by sperm chromatin structure assay (SCSA). This was in contrast to the results presented by Volpes and colleagues. Their group found a higher proportion of sperm DFI in DSU-separated sperm compared with DGC-SU-separated sperm by using sperm chromatin dispersion (SCD) test [5]. The inconsistency of the results between the present and previous studies may result from different assessment methods of sperm DNA fragmentation.

In addition to the evaluation of sperm quality after DSU treatment, we also compared ART outcomes of DSU group with DGC-SU group. We reported herein that DSU technique did not show any significant difference in all ART outcomes as compared to the DGC-SU technique. However, Our results were inconsistent with the findings of Palini and colleagues in ICSI cycles [20]. Their results stated that blastulation rates per D3 embryos or per fertilized oocyte and pregnancy rates in DSU group were

significant higher when compared to DGC-SU group. This inconsistency may due to additional sperm selection, which would be performed in the process of ICSI insemination. Recently, it was reported that the clinical outcomes were not significantly different between the DGC-SU and conventional pellet swim-up groups [43]. This finding is in agreement with our results. However, lower fertilization rates were shown in our study. This may attribute to the fact that we moved into a new IVF laboratory at the end of 2015. Increase in Volatile organic compounds (VOCs) concentration of a new IVF lab had effects on IVF outcomes such as fertilization [44].

Several studies have reported that bacterial contamination can lead to compromised sperm quality and suboptimal fertilization rate [45–47]. Therefore, we have to take into consideration the potential bacterial contamination in DSU-separated sperm in this study. Bacterial contamination was only detected in one out of 88 upper layer medium samples and no bacteria growing in simulating fertilization experiment medium samples from DSU group, although bacterial contamination were positive in 64 of 92 native semen samples. These data suggest that DSU treatment did not lead to higher risk of bacterial contamination than DGC-SU treatment.

Another reason in support of DSU technique is the reduction of execution time and labor of semen treatment relative to DGC-SU technique. Clearly, the DSU technique does not require redundant density gradients. However, DGC, but not DGC-SU is the most common technique used for the isolation of normal spermatozoa in IVF laboratories worldwide. Subsequently, we need to compare DSU technique with DGC technique to make our conclusions more convincing. Moreover, we utilized DSU technique for IVF sperm preparation depending on post-DSU sperm concentration. If the concentration of motile sperm after DGC was not enough for IVF insemination, additional centrifugation should be performed. It means that different IVF populations have their own optimal method of sperm preparation.

Leukocytes represent the predominant source of ROS. Both swim-up and DGC removed most seminal leukocytes. Swim-up should be preferred for sperm preparation in the leukocytospermic patients because it decreases leukocyte concentration more than DGC [48]. This may be the reason why a combination technique of DGC and swim-up is widely used to prepare sperm for IVF insemination in China. However, it was controversial on the effect of seminal leukocytes on ART outcomes. Some studies have suggested that leukocytes negatively affect the outcome of both IVF and ICSI [49–50]. Conversely, it has been observed that leukocytes do not always have a negative effect on semen quality and ART outcomes [51–52]. Leukocytospermic patients with infection-free were also included in the present study. We cannot exclude that the results of this study may extend to leukocytospermic patients. A study specifically addressed to this purpose should be designed. Recently, it has been reported that up to 30% of male partners of infertile couples are positive for C. trachomatis [53]. It is of great significance to investigate which method of sperm preparation is most suitable for patients with C. trachomatis infections. However, the whole IVF patients were negative for C. trachomatis in our data, and the results of this study cannot be extrapolated to patients with C. trachomatis infections.

Conclusions

In summary, we found that the DSU technique is comparable to the DGC-SU technique on sperm preparation for IVF insemination in terms of recovery of progressive motile spermatozoa with DNA integrity, safety of operation and ART outcomes. However, the DSU technique requires less time and labor for sperm preparation in comparison to the DGC-SU technique. This study provided evidence that the DGC-SU technique can be replaced with the DSU technique in IVF treatments.

Abbreviations

AR: Acrosomal reaction

BMI: Body-mass index

DGC-SU: Density-gradient centrifugation followed by the swim-up

DSU: Direct swim-up without centrifugation

DFI: DNA fragmentation index

FSH: Follicle-stimulating hormone

HDS: High DNA stainable

ICSI: Intracytoplasmic sperm injection

LH: Luteinizing hormone

MMP: Mitochondrial membrane potential

2PN: two pronuclei

Declarations

Ethics approval and consent to participate

Due to the retrospective nature of the study, informed consent was waived. However, the study was approved by Ethics Committees on Human Research of the First Affiliated Hospital of University of Science and Technology of China.

Consent for publication

Not applicable.

Availability of data and materials

The primary data for this study were available from patient medical records.

Competing interests

The authors declare that there are no conflicts of interest.

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Authors' contributions

X.-H. T. was responsible for conception and design of the study. F.-T. L. performed the statistical analyses and writing the manuscript. L.-M. W., R.-T. J., B. X., N.-R.ZH. and J.-Y. L. carried out experimental work. Y.-S. L. contributed to revising and editing the manuscript. All authors approved of the final version to be published.

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References

1. Ajduk A, Zernicka-Goetz M. Quality control of embryo development. Mol Aspects Med. 2013;34(5):903–18.
2. Avendano C, Franchi A, Duran H, Oehninger S. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril. 2010;94(2):549–57.
3. Simon L, Murphy K, Shamsi MB, Liu L, Emery B, Aston KI, et al. Paternal influence of sperm DNA integrity on early embryonic development. Hum Reprod. 2014;29(11):2402–12.
4. Yamanaka M, Tomita K, Hashimoto S, Matsumoto H, Satoh M, Kato H, et al. Combination of density gradient centrifugation and swim-up methods effectively decreases morphologically abnormal sperms. J Reprod Dev. 2016;62(6):599–606.
5. Volpes A, Sammartano F, Rizzari S, Gullo S, Marino A, Allegra A. The pellet swim-up is the best technique for sperm preparation during in vitro fertilization procedures. J Assist Reprod Genet. 2016;33(6):765–70.

6. Bormann CL, Roche A, Hassun P, Motta E, Sarafini P, Smith GD. The effect of sperm separation on sperm chromatin decondensation and motility at 0 and 24 hours of culture. Fertil Steril. 2009;92(3):S144.
7. Ghumman S, Adiga SK, Upadhya D, Kalthur G, Jayaraman V, Rao SB, et al. Combination of swim-up and density gradient separation methods effectively eliminate DNA damaged sperm. J Turk Ger Gynecol Assoc. 2011;12(3):148–52.
8. Aitken RJ, Bronson R, Smith TB, De Iuliis GN. The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. Mol Hum Reprod. 2013;19(8):475–85.
9. Wang M, Sun J, Wang L, Gao X, Lu X, Wu Z, et al. Assessment of density gradient centrifugation (DGC) and sperm chromatin dispersion (SCD) measurements in couples with male factor infertility undergoing ICSI. J Assist Reprod Genet. 2014;31(12):1655–63.
10. Zini A, Nam RK, Mak V, Phang D, Jarvi K. Influence of initial semen quality on the integrity of human sperm DNA following semen processing. Fertil Steril. 2000;74(4):824–7.
11. Muratori M, Maggi M, Spinelli S, Filimberti E, Forti G, Baldi E. Spontaneous DNA fragmentation in swim-up selected human spermatozoa during long term incubation. J Androl. 2003;24(2):253–62.
12. Oguz, Y., I. Guler, A. Erdem, M. F. Mutlu, S. Gumuslu, M. Oktem, N. Bozkurt, and M. Erdem, The effect of swim-up and gradient sperm preparation techniques on deoxyribonucleic acid (DNA) fragmentation in subfertile patients. J Assist Reprod Genet, 2018. 35(6): 1083–9.
13. Kim, S. W., B.C. Jee, S. K. Kim, and S. H. Kim, Sperm DNA fragmentation and sex chromosome aneuploidy after swim-up versus density gradient centrifugation. Clin Exp Reprod Med, 2017. 44(4): 201–6.
14. Gonzalez-Marin C, Gosalvez J, Roy R. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci. 2012;13(11):14026–52.
15. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod. 2012;27(10):2908–17.
16. Simon L, Brunborg G, Stevenson M, Lutton D, McManus J, Lewis SE. Clinical significance of sperm DNA damage in assisted reproduction outcome. Hum Reprod. 2010;25(7):1594–608.
17. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21(11):2876–81.
18. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2008;23(12):2663–8.
19. Aitken RJ, Finnie JM, Muscio L, Whiting S, Connaughton HS, Kuczera L, et al. Potential importance of transition metals in the induction of DNA damage by sperm preparation media. Hum Reprod. 2014;29(10):2136–47.

20. Palini S, Stefani S, Primiterra M, Benedetti S, Barone S, Carli L, et al. Comparison of in vitro fertilization outcomes in ICSI cycles after human sperm preparation by density gradient centrifugation and direct micro swim-up without centrifugation. JBRA Assist Reprod. 2017;21(2):89–93.
21. Palini S, Primiterra M, De Stefani S, Pedna MF, Sparacino M, Farabegoli P, et al. A new micro swim-up procedure for sperm preparation in ICSI treatments: preliminary microbiological testing. JBRA Assist Reprod. 2016;20(3):94–8.
22. Li Z, Zhou Y, Liu R, Lin H, Liu W, Xiao W, et al. Effects of semen processing on the generation of reactive oxygen species and mitochondrial membrane potential of human spermatozoa. Andrologia. 2012;44(3):157–63.
23. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod. 1999;14(4):1039–49.
24. Bungum, M., P. Humaidan, M. Spano, K. Jepson, L. Bungum, and A. Giwercman, The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod, 2004. 19(6): 1401–8.
25. Tong XH, Wu LM, Jin RT, Luo LH, Luan HB, Liu YS. Fertilization rates are improved after IVF if the corona radiata is left intact in vitrified-warmed human oocytes. Hum Reprod. 2012;27(11):3208–14.
26. Tong XH, Wu LM, Jin RT, Luan HB, Liu YS. Human oocyte vitrification with corona radiata, in autologous follicular fluid supplemented with ethylene glycol, preserves conventional IVF potential: birth of four healthy babies. Reprod Fertil Dev. 2014;26(7):1001–6.
27. Chen L, Xu Z, Zhang N, Wang B, Chen H, Wang S, et al. Neonatal outcome of early rescue ICSI and ICSI with ejaculated sperm. J Assist Reprod Genet. 2014;31(7):823–8.
28. Zegers-Hochschild, F., G. D. Adamson, S. Dyer, C. Racowsky, J. de Mouzon, R. Sokol, L. Rienzi, A. Sunde, L. Schmidt, I. D. Cooke, J. L. Simpson, and S. van der Poel, The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017. 108(3): 393–406.
29. Zheng, W. W., G. Song, Q. L. Wang, S. W. Liu, X. L. Zhu, S. M. Deng, A. Zhong, Y. M. Tan, and Y. Tan, Sperm DNA damage has a negative effect on early embryonic development following in vitro fertilization. Asian J Androl. 2018. 20(1): 75–9.
30. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril. 1992;57(2):409–16.
31. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010;93(4):1027–36.
32. Twigg J, Irvine DS, Houston P, Fulton N, Michael L, Aitken RJ. Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma. Mol Hum Reprod. 1998;4(5):439–45.
33. O'Connell M, McClure N, Powell LA, Steele EK, Lewis SE. Differences in mitochondrial and nuclear DNA status of high-density and low-density sperm fractions after density centrifugation preparation. Fertil Steril. 2003;79 Suppl 1:754–62.

34. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod. 2007;22(1):174–9.
35. Holden, C. A., R. V. Hyne, A. H. Sathananthan, and A. O. Trounson, Assessment of the human sperm acrosome reaction using concanavalin A lectin. Mol Reprod Dev, 1990. 25(3): 247–57.
36. Zhang, G., Z. Wang, X. Ling, P. Zou, H. Yang, Q. Chen, N. Zhou, L. Sun, J. Gao, Z. Zhou, J. Cao, and L. Ao, Mitochondrial Biomarkers Reflect Semen Quality: Results from the MARCHS Study in Chongqing, China. PLoS One, 2016. 11(12): e0168823.
37. Marchetti C, Obert G, Deffosez A, Formstecher P, Marchetti P. Study of mitochondrial membrane potential, reactive oxygen species, DNA fragmentation and cell viability by flow cytometry in human sperm. Hum Reprod. 2002;17(5):1257–65.
38. Katsuki T, Hara T, Ueda K, Tanaka J, Ohama K. Prediction of outcomes of assisted reproduction treatment using the calcium ionophore-induced acrosome reaction. Hum Reprod. 2005;20(2):469–75.
39. Ozmen B, Caglar GS, Koster F, Schopper B, Diedrich K, Al-Hasani S. Relationship between sperm DNA damage, induced acrosome reaction and viability in ICSI patients. Reprod Biomed Online. 2007;15(2):208–14.
40. Ghaleno LR, Valojerdi MR, Janzamin E, Chehrazi M, Sharbatoghli M, Yazdi RS. Evaluation of conventional semen parameters, intracellular reactive oxygen species, DNA fragmentation and dysfunction of mitochondrial membrane potential after semen preparation techniques: a flow cytometric study. Arch Gynecol Obstet. 2014;289(1):173–80.
41. Birks AG, Izzard H, Morroll DR, Prior JR, Troup SA, Lieberman BA, et al. The routine assessment of sperm motility at room temperature and 37 degrees C. Int J Androl. 1994;17(6):289–91.
42. Bonato M, Cornwallis CK, Malecki IA, Rybnik-Trzaskowska PK, Cloete SW. The effect of temperature and pH on the motility and viability of ostrich sperm. Anim Reprod Sci. 2012;133(1–2):123–8.
43. Kim EK, Kim EH, Kim EA, Lee KA, Shin JE, Kwon H. Comparison of the effect of different media on the clinical outcomes of the density-gradient centrifugation/swim-up and swim-up methods. Clin Exp Reprod Med. 2015;42(1):22–9.
44. Khoudja, R. Y., Y. Xu, T. Li, and C. Zhou, Better IVF outcomes following improvements in laboratory air quality. J Assist Reprod Genet, 2013. 30(1): 69–76.
45. Shalika S, Dugan K, Smith RD, Padilla SL. The effect of positive semen bacterial and Ureaplasma cultures on in-vitro fertilization success. Hum Reprod. 1996;11(12):2789–92.
46. Yaniz JL, Marco-Aguado MA, Mateos JA, Santolaria P. Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 degrees C. Anim Reprod Sci. 2010;122(1–2):142–9.
47. Burrello N, Calogero AE, Perdichizzi A, Salmeri M, D'Agata R, Vicari E. Inhibition of oocyte fertilization by assisted reproductive techniques and increased sperm DNA fragmentation in the presence of Candida albicans: a case report. Reprod Biomed Online. 2004;8(5):569–73.

48. Ricci, G., S. Perticarari, R. Boscolo, R. Simeone, M. Martinelli, L. Fischer-Tamaro, S. Guaschino, and G. Presani, Leukocytospermia and sperm preparation—a flow cytometric study. Reprod Biol Endocrinol, 2009. 7: 128.
49. Yilmaz, S., M. Koyuturk, G. Kilic, O. Alpak, and A. Aytoz, Effects of leucocytospermia on semen parameters and outcomes of intracytoplasmic sperm injection. Int J Androl, 2005. 28(6): 337–42.
50. Vicino, M., G. Loverro, S. Simonetti, L. Mei, and L. Selvaggi, The correlation between idiopathic leukocytospermia, embryo quality and outcome in the FIVET and ICSI procedures. Minerva Ginecol, 1999. 51(11): 413–20.
51. Ricci, G., M. Granzotto, S. Luppi, E. Giolo, M. Martinelli, G. Zito, and M. Borelli, Effect of seminal leukocytes on in vitro fertilization and intracytoplasmic sperm injection outcomes. Fertil Steril, 2015. 104(1): 87–93.
52. Lackner, J. E., I. Mark, K. Sator, J. Huber, and M. Sator, Effect of leukocytospermia on fertilization and pregnancy rates of artificial reproductive technologies. Fertil Steril, 2008. 90(3): 869–71.
53. Vigil, P., P. Morales, A. Tapia, R. Riquelme, and A.M. Salgado, Chlamydia trachomatis infection in male partners of infertile couples: incidence and sperm function. Andrologia, 2002. 34(3): 155–61.

Tables

Table 1 Comparison of sperm classical parameters and quality indicators between the DSU and DGC-SU group.

Characteristics	native semen (n=37)	DSU (n=37)	DGC-SU (n=37)	*p*-value			
				Among three groups	DSU vs. DGC-SU	Naive vs. DSU	Naive vs. DGC-SU
Sperm concentration	43.13±24.69	1.55±0.95	1.41±0.96	<0.001	0.965	<0.001	<0.001
Progressive motility (%)	23.90±9.06	68.37±13.98	60.85±17.65	<0.001	**0.023**	<0.001	<0.001
Sperm DFI (%)	22.56±13.67	9.04±19.87	9.42±22.82	0.026	0.944	0.017	0.020
Sperm HDS (%)	8.08±3.82	3.70±4.10	2.22±2.23	<0.001	0.146	<0.001	<0.001
Sperm AR (%)	18.42±11.93	45.01±29.76	43.01±22.05	0.001	0.784	0.001	0.001
Sperm MMP (%)	41.22±18.15	81.38±23.78	86.53±22.10	<0.001	0.409	<0.001	<0.001

HDS: High DNA stainable; AR: Acrosomal reaction; MMP: Mitochondrial membrane potential; Data were presented as mean ± standard deviation (SD); *p*-values <0.05 were considered statistically significant.

Table 2 Demographic characteristics of IVF patients between the DSU and DGC-SU group.

	DSU	DGC-SU	DSU vs DGC-SU *p*-value
No. of cycles	536	687	
Male age (years)	32.10±4.97	32.28±5.14	0.543
Female age (years)	30.56±4.22	30.58±4.21	0.944
Female BMI (kg/m^2)	22.02±2.77	22.20±2.80	0.267
Infertility duration (years)	3.32±2.39	3.53±2.43	0.149
Indication of IVF			
Tubal factor (%)	57.46 (308/536)	58.22 (400/687)	0.789
Endometriosis (%)	5.78 (31/536)	4.37 (30/687)	0.367
Multifactorial (%)	27.43 (147/536)	28.22 (198/687)	0.590
Unexplained (%)	9.32 (50/536)	8.59 (59/687)	0.652
Basal FSH (IU/L)	7.13±1.82	7.04±1.83	0.505
Basal estradiol (pg/mL)	67.71±141.74	57.66±72.74	0.200
Basal LH(IU/L)	5.42±3.31	5.08±4.35	0.246

DSU: Direct swim-up without centrifugation; DGC-SU: Density-gradient centrifugation followed by swim-up; DFI: DNA fragmentation index;

BMI: Body-mass index; FSH: Follicle-stimulating hormone; LH: luteinizing hormone; Data were presented as mean ± standard deviation (SD)

Table 3 Comparison of laboratory outcomes between the DSU and DGC-SU group.

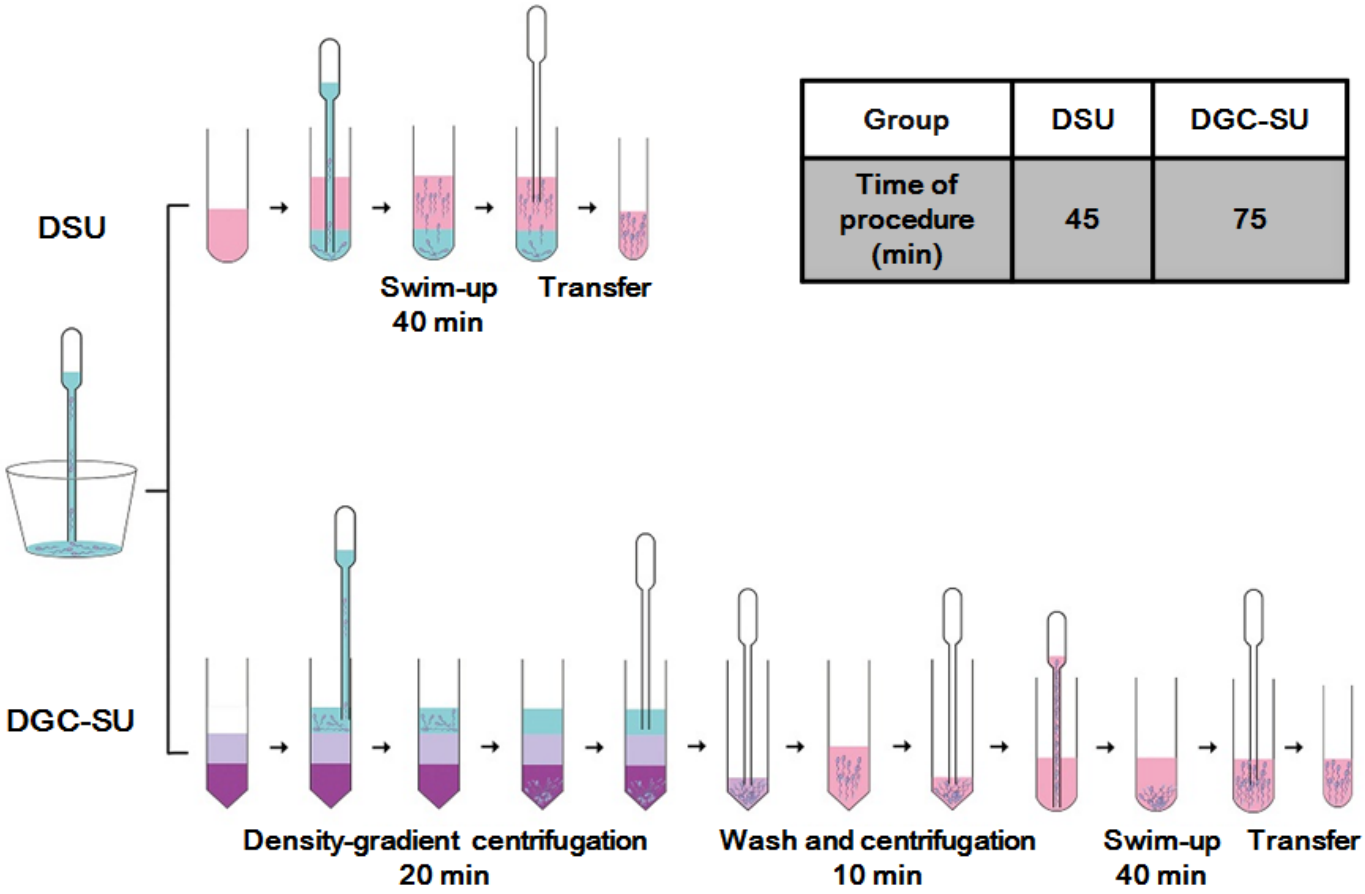
	DSU	DGC-SU	DSU vs DGC-SU *p*-value
No. of cycles	536	687	
No. of retrieved oocytes	12.22±6.87	12.37±6.89	0. 719
No. of 2PN	7.72±4.65	7.90±4.52	0.482
Fertilization rate (2PN, %)	63.14 (4137/6552)	63.91 (5430/8496)	0.330
Rescue ICSI rate (%)	7.46 (40/536)	6.84 (47/687)	0.675
Cleavage rate (%)	98.72 (4084/4137)	98.66 (5357/5430)	0.788
High-quality embryo rate (%)	68.24 (2787/4084)	67.56 (3619/5357)	0.480

2PN: two pronuclei; ICSI: intracytoplasmic sperm injection; Data were presented as mean ± standard deviation (SD) or percent (%).

Table 4 Comparison of pregnancy outcomes between the DSU and DGC-SU group.

	DSU	DGC-SU	DSU vs DGC-SU *p*-value
No. of embryo transfer cycles	227	310	
No. of embryos per transfer (mean± SD)	1.90±0.30	1.93±0.25	0.168
Chemical pregnancy rate (%)	59.03 (134/227)	62.25 (193/310)	0.449
Clinical pregnancy rate (%)	52.86 (120/227)	53.87 (167/310)	0.817
Implantation rate (%)	34.88 (150/432)	35.50 (213/600)	0.796
Spontaneous abortion rate (%)	7.46 (10/134)	7.25 (14/193)	0.712
Take-home baby rate (%)	46.25(105/227)	46.45 (144/310)	0.964

Figures



Group	DSU	DGC-SU
Time of procedure (min)	45	75

Figure 1

Schematic diagram of sperm preparation with direct swim-up without centrifugation (DSU) and density-gradient centrifugation followed by swim-up (DGC-SU) technique

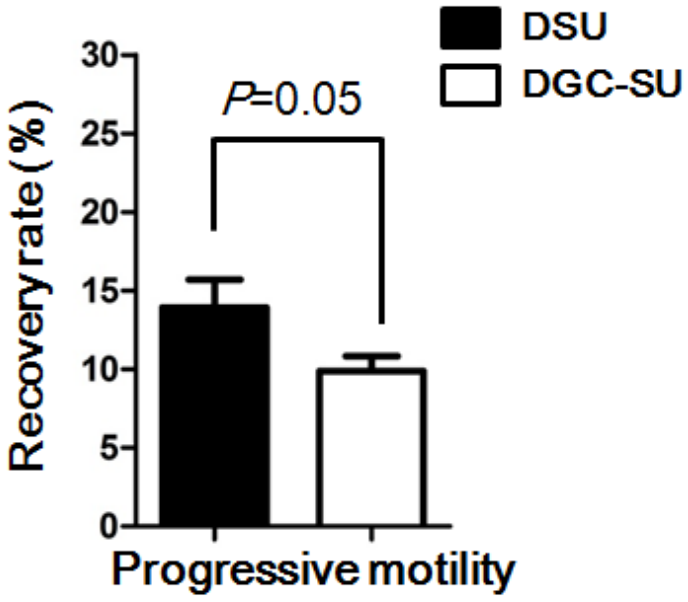


Figure 2

Comparison of recovery rate of sperm with progressive motility between DSU and DGC-SU technique The recovery rate of progressive motility was calculated as the number of separated sperm with progressive motility divided by the number of native sperm with progressive motility (n=37). Data are shown as the mean ± S.D. p-value <0.05 was considered statistically significant.

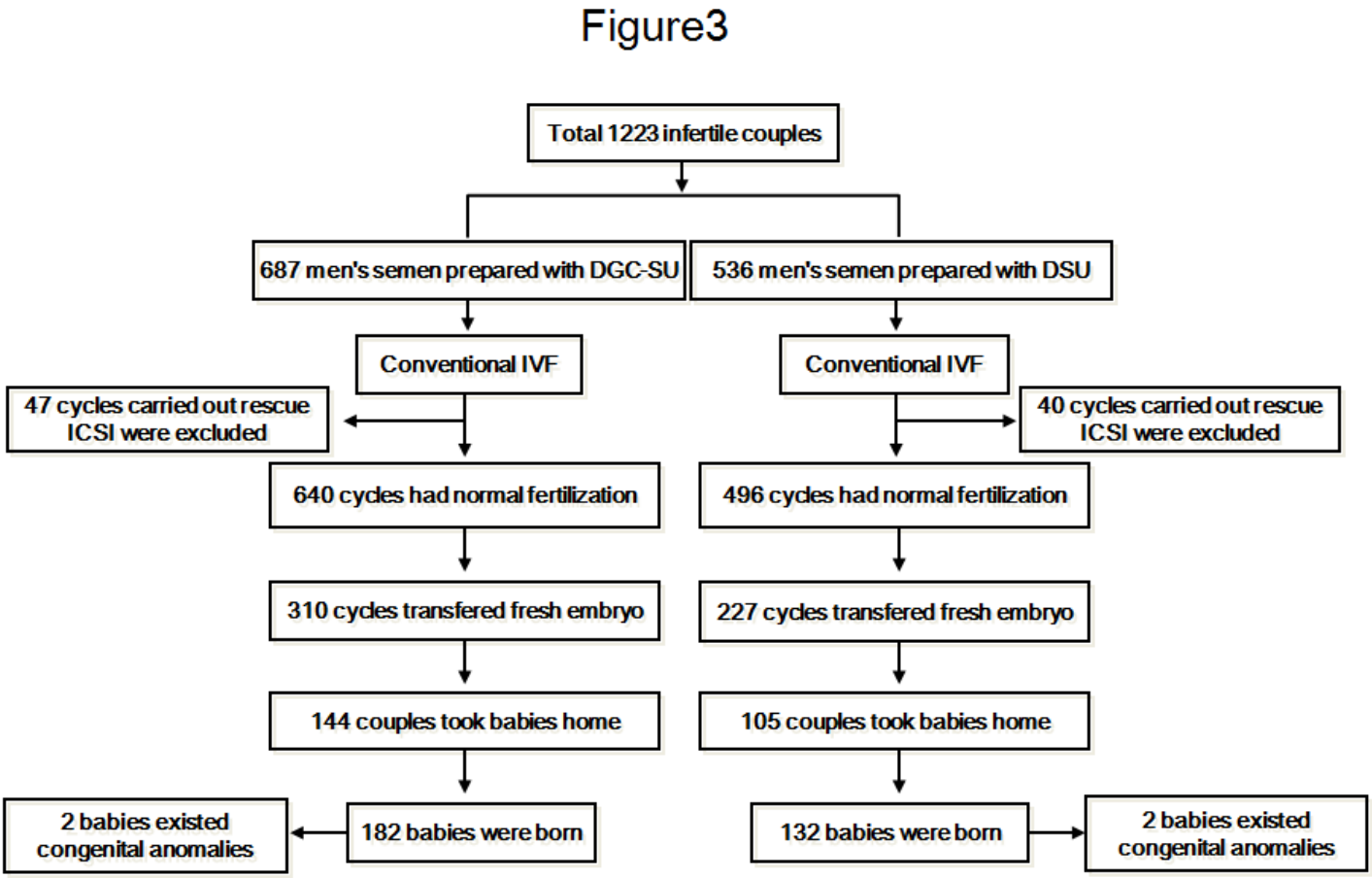


Figure 3

Flowchart reviewing ART outcomes of conventional IVF cycles with the DSU and DGC-SU technique

Supplementary Files

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- supplementarytable.docx