Positive rheotaxis extended drop: a one-step procedure to select and recover sperm with mature chromatin for intracytoplasmic sperm injection

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Abstract

Purpose The purpose of this study was to develop a novel one-step ICSI approach to select sperm with better chromatin maturity than the conventional method.

Methods This was a pilot diagnostic study, which prospectively recruited men during a 6-month period in a University-affiliated infertility centre. Forty consecutive semen samples were provided for analysis. The positive rheotaxis extended drop (PRED) was set up creating a pressure and viscosity gradient. Each semen sample was divided into four aliquots: one aliquot for density gradient centrifugation (DGC), two aliquots for PRED (fresh semen (PRED-FS) and processed semen (PRED-DGC)), and one aliquot as the control (FS). In PRED, a mean of 200 spermatozoa were collected consecutively without selection from the outlet reservoir. The aniline blue assay was used to assess chromatin immaturity.

Results The mean channel length, measured from inlet to outlet, was 32.55 ± 0.86 mm, with a mean width of 1.04 ± 0.21 mm. In 82.5% of cases (33/40), at least 50

spermatozoa were captured between 15 and 30 min. Improved chromatin maturity after the DGC preparation and the PRED approach was observed in all samples. This was reflected by a mean reduction from $28.65 \pm 8.97\%$ uncondensed chromatin in the native ejaculates to $17.29 \pm 7.72\%$ in DGC and $0.89 \pm 1.31\%$ in the PRED approach ($P < 0.01$).

Conclusions The PRED method may improve the current ICSI technique by providing it with its own sperm selection process. ICSI would probably become an even more complete technique comprising selection, capture and injection of the male gamete.

Keywords Intracytoplasmic sperm injection · Men · Microfluidics · Spermatozoa · Sperm chromatin

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Introduction

Mammalian spermatozoa undergo an intense selection process during their migration through the female reproductive tract [1]. Indeed, spermatozoa exert a substantial effort due to the long migration distance relative to their size ($\sim 100 \mu\text{m}$). These cells may swim approximately 1000 times their length [2] and cross highly viscous fluid with many immune cells, which may inactivate a significant portion of them. Therefore, few spermatozoa traverse the uterine cavity and travel to the tubal ampulla for oocyte fertilisation [3]. This natural process is important for the selection of the spermatozoon most fit for fecundation.

In the last decades, intracytoplasmic sperm injection (ICSI) has become the most important technique in assisted human reproduction to help overcome male infertility. However, this technique bypasses the natural selection barriers because the

selective pressure in the female reproductive tract is superseded by the random choice of an embryologist [4]. Therefore, ICSI has been a process of “choice”, not a process of “selection”. Consequently, a low-quality male gamete with compromised nuclear and cytoplasmic competence may be chosen [5–10], which could result in fertilisation failure, impaired embryo development, reduced implantation or pregnancy rate and increased miscarriage rates [11, 12].

Techniques for the selection of spermatozoa used in ICSI were designed in order to obtain gametes with ideal morphology and motility. However, fertilisation and pregnancy rates could be affected by nuclear anomalies that would remain undetectable by embryologists during the standard sperm selection procedure. This is a great concern with regard to obtaining a good-quality embryo without DNA problems. Mimicking the natural mechanisms of selection observed in mammals, including swimming through viscous liquids and against a fluid flow (positive rheotaxis), could reduce the quantity of low-quality spermatozoa [13]. Such efforts during migration may select spermatozoa with better morphology and healthy mitochondria, improving the chances of choosing a better male gamete [14].

Microfluidic technology may be a novel alternative to spermatozoa selection that can improve the results of conventional methods [15–19]. Recently, a microfluidic sperm sorter (MFSS) described by Shirota et al. was able to reduce the risk of selecting a spermatozoon with DNA fragmentation to less than 1% [20]. Thus, if microfluidic features could be incorporated into ICSI dishes, this technique would have its own sperm selection process, which could improve its efficiency while reducing time and process costs.

In this preliminary study, we tested a novel approach for spermatozoa selection using a modified ICSI dish inspired by microfluidic technology. The primary goal of our work was to establish an approach to rapidly and effectively isolate functional spermatozoa in a standard ICSI dish, without previous semen processing. We assessed sperm maturity in samples from unselected males and evaluated whether the positive rheotaxis extended drop (PRED) method allowed for more effective recovery of sorted motile sperm with mature chromatin than the density gradient centrifugation (DGC) procedure.

Materials and methods

Study population and semen analysis

Men presenting to the human reproduction centre at our institution for infertility assessment between July and December 2014 were invited to participate in this study ($n = 40$). Semen samples were obtained after a recommended 2–5 days of sexual abstinence. Following liquefaction (30 min), all samples

were subjected to a conventional light microscopic analysis to determine semen volume, sperm concentration, total sperm output, normal morphology and motility according to the World Health Organization recommendations [21]. Semen samples with a severely low sperm count ($< 1 \times 10^6/\text{ml}$) were excluded from the study.

PRED

For this procedure, a few droplets of medium (Modified HTF, Irvine Scientific, Santa Ana, CA, USA) supplemented with a 10% synthetic serum substitute (SSS, Irvine Scientific, Santa Ana, CA, USA) were prepared on a standard Falcon 1006 Petri dish (Becton Dickinson, Franklin Lakes, NJ, USA) at 37 °C. The droplets were connected to each other by bridges of medium created with a pipette tip and were covered with mineral oil (Irvine Scientific, Santa Ana, CA, USA).

The PRED consisted of two culture medium reservoirs, a pressure control reservoir (PCR) and a semen input reservoir (SIR) linked by a channel (Fig. 1).

The droplets of culture medium were added to the dish with precise spacing and sequencing to obtain a pressure difference between the reservoirs. To facilitate this task, the dish was virtually divided into four sections by a vertical and a horizontal axis, as shown in Fig. 2. The first droplet added was the central droplet (droplet 1) at the intersection of the two axes. Another five droplets were added based on the central droplet position, resulting in the layout shown in step 1 (Fig. 2, step 1).

The next step was to assemble the reservoirs. Droplets 5 and 6 were added to the SIR via a series of resizing and merging steps as shown in Fig. 3 (steps 2a–2d). The PCR was obtained by merging the central droplet with droplet 4 (Fig. 3, step 2e). The final configuration of the reservoirs was completed at the end of step 2 (Fig. 3, step 2).

In step 3, the PCR and the SIR were connected by a narrow channel (± 1 mm). First, the lower edge of the PCR was extended (Fig. 4, step 3a). Then, a channel was formed starting at the lower edge of the SIR and merging with the lower edge of the PCR, forming a 90° angle (Fig. 4, step 3b). The connection of the reservoirs was completed at the end of step 3 (Fig. 4, step 3).

The next task was to transfer the contents of droplet 3 to the PCR, which increased the pressure in this reservoir. Then, 10 μl of polyvinylpyrrolidone (PVP) solution (PVP, Irvine Scientific, Santa Ana, CA, USA) was added to the mark left by droplet 3. Next, the lower edge of droplet 2 was merged with the upper edge of the PVP drop (former droplet 3). To complete the circuit, the upper edge of the PCR was merged with the lower edge of the PVP drop. A new pipette tip was then used to transfer 10 μl of culture medium from the SIR to the PCR to increase the pressure. Using the same tip, we added 5–10 μl of semen sample to the SIR, with variable motile sperm concentration according to sample characteristics. Another pipette tip

Fig. 1 PRED: positive rheotaxis extended drop

was used to add the egg droplets, and then the dish was covered with mineral oil (Fig. 5, step 4). The spermatozoa migrated from the SIR to the PCR, where they were captured. A single experienced embryologist performed all steps of the procedure. As this is a novel method that requires only basic infertility laboratory skills, no specific training was necessary prior to initiation of this study. Except for the addition of the semen sample, the whole procedure can be viewed in the Supplementary Video 1, which can be safely and accurately performed in about 2 min.

General protocol

After initial analysis, each semen sample was divided into two fractions. The first fraction of native sperm (Fraction A) was not treated and was divided into two aliquots. One aliquot (10 μ l) was smeared onto a glass slide for posterior analysis of sperm maturity (raw fresh semen, R-FS), and the other

aliquot was subjected to the PRED procedure (PRED fresh semen, PRED-FS).

The second fraction (Fraction B) was prepared by double-DGC (Isolate, Irvine Scientific, Santa Ana, CA, USA). Samples were loaded onto a 45 and a 90% discontinuous gradient and centrifuged at 300 $\times$ g for 20 min at room temperature (25 $^{\circ}$ C). The resulting 90% pellet was washed by centrifugation for an additional 10 min and resuspended in 0.5 ml of HEPES-HTF medium.

After DGC, the resulting suspension was subsequently divided into two aliquots: the first aliquot was placed into a 7% PVP solution droplet on a conventional ICSI dish (ICSI density gradient centrifugation, ICSI-DGC), and the other was placed into a PRED dish (PRED density gradient centrifugation, PRED-DGC).

To evaluate the sperm maturity of each sample (PRED-FS, PRED-DGC and ICSI-DGC), we used the conventional ICSI procedure to consecutively collect a mean of 200 spermatozoa from the PVP drop with an injection pipette (ICSI

Fig. 2 Initial positioning of the droplets. Droplet 1: central droplet. Droplet 2: droplet positioned at 2/3 of the distance between the central droplet and the upper edge of the dish. Droplet 3: droplet positioned at half the distance between the central droplet and droplet 2. Droplet 4: droplet positioned below the central droplet conforming to the same distance between the droplets placed above. Droplet 5: droplet positioned at

half the distance between the central droplet and the right edge of the dish, along the horizontal axis. Droplet 6: droplet positioned at half the distance between droplet 5 and the right edge of the dish, along the horizontal axis. Step 1: Illustrative picture of the system at the end of step 1

Fig. 3 Assembling the reservoirs PCR (pressure control reservoir) and SIR (semen input reservoir). Step 2a: resizing droplet 5 by extending its upper edge. Step 2b: resizing droplet 6 and merging its upper edge with droplet 5. Step 2c: resizing droplet 5 by extending its lower edge. Step 2d: resizing droplet 6 and merging its lower edge with droplet 5, thereby completing the formation of the SIR. Step 2e: merging the central droplet with droplet 4 to form the PCR. Step 2: Illustrative picture of the system at the end of step 2

Micropipettes, Origio, Måløv, Denmark) and place them in a 6- μ l drop of distilled water on a glass slide previously marked with a circle. The time required for the selection step was 15–45 min/sample.

Aniline blue-eosin staining

Spermatozoa were fixed with 95% ethanol for 5 min [22], followed by staining with a 5% aniline blue (Sigma-Aldrich Co., St. Louis, MO, USA) solution in 4% acetic acid (pH 3.5) for 5 min. After washing, the samples were stained with a 0.5% eosin (Merck) solution for 1 min and dried. Sperm were classified as immature if the head stained dark blue and were classified as mature if the head stained red-pink [23, 24]. In the R-FS group, 200 spermatozoa were counted in different areas of each slide. In the remaining groups (PRED-FS, PRED-DGC and ICSI-DGC), all spermatozoa were counted. The spermatozoa were evaluated under light microscopy at $\times 1000$ magnification. The percentage of abnormal sperm chromatin condensation was calculated as the ratio of the

number of dark blue sperm to the total number of sperm analysed.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used to verify the normal or non-normal distribution of values. The non-parametric Wilcoxon test was used to compare matched series. The efficiency of each method (ICSI-DGC, PRED-FS and PRED-DGC) was defined by determining the relative reduction in the percentage of aniline blue staining in relation to control or raw semen samples (R-FS). This was calculated by subtracting the mean percentage of aniline blue staining in the processed group from the mean percentage of aniline blue staining in the control group, dividing by the mean percentage of aniline blue staining in the control group, and then multiplying by 100. SPSS version 22.0 for Windows (SPSS® Inc.; Illinois, USA) was used to analyse the data. Differences were considered significant if $P < 0.05$ and highly significant if $P < 0.01$.

Fig. 4 Formation of the channel between the PCR (pressure control reservoir) and the SIR (semen input reservoir). Step 3a: resizing of the PCR by extending its lower edge. Step 3b: bridge formation between the lower edge of the PCR and the lower edge of the SIR. Step 3: Illustrative picture of the system at the end of step 3

Fig. 5 Completion of the circuit and addition of egg droplets. Step 4a: merging of droplet 1 with droplet 2 (which contains PVP—polyvinylpyrrolidone) and addition of the seminal sample into the SIR. Step 4b: completion of the circuit and addition of egg droplets. Step 4: Illustrative picture of the system at the end of step 4

Compliance with ethical statements

All subjects provided written informed consent for participation in this study. This project was conducted in accordance with the Declaration of Helsinki for research involving human subjects and received approval from our Institutional Review Board.

Results

A total of 40 men participated in this prospective evaluation, which addressed the efficiency of the PRED-ICSI approach in selecting highly motile, mature spermatozoa for ICSI purposes. The baseline characteristics of the study population are shown in Table 1. The mean age was 34.23 ± 7.42 years (range 20–48 years). The mean sperm concentration was $56.69 \pm 46.29 \times 10^6/\text{ml}$; the corresponding percentage of progressive motile spermatozoa (WHO grade a and b) was $29.08 \pm 17.49\%$, and normal sperm morphology was $12.11 \pm 8.93\%$.

The mean channel length measured from the inlet to the outlet was 32.55 ± 0.86 mm, with a mean width of 1.04 ± 0.21 mm. The PRED design was able to maintain the desired flow direction for at least 45 min. In 82.5% of cases (33/40), at least 50 spermatozoa were captured between 15 and 30 min. In only 5 cases (12.5%) were all spermatozoa captured between 30 and 45 min. Food dyes were used to demonstrate the fluid dynamics and the safety of the procedure, as shown in Fig. 6, supplementary videos 2 and 3.

A positive correlation was found between the ejaculated sperm count and the percentage of sperm captured before 30 min with the PRED approach ($R^2 = 62\%$, $P < 0.001$). Table 2 shows the percentages of morphologically normal spermatozoa in semen and spermatozoa recovered by the PRED method and DGC. We observed a higher percentage of sperm sorted by the DGC preparation (ICSI-DGC), and the PRED approach (PRED-FS and PRED-DGC) were morphologically normal compared to R-FS. A significant improvement ($P < 0.01$) was also observed when comparing the results

obtained after the DGC preparation and the PRED approach. Sperm sorted by PRED-FS showed similar morphology to those obtained by PRED when processed semen was used (PRED-DGC).

Regarding chromatin immaturity, 100% of men had better results after the DGC preparation and the PRED approach (Table 2). This was reflected by a mean reduction from $28.65 \pm 8.97\%$ of uncondensed chromatin in the native ejaculates to $17.29 \pm 7.72\%$ in DGC-processed semen ($P < 0.01$). Even greater reductions were achieved when the PRED approach was used, with means of $0.89 \pm 1.31\%$ (PRED-FS) and $1.05 \pm 1.63\%$ (PRED-DGC) of uncondensed chromatin compared to the DGC-processed sample ($P < 0.01$). No significant difference was found between PRED-FS and PRED-DGC results ($P = 0.25$).

Figure 7 demonstrates the ability of the DGC preparation and the PRED approach to select mature sperm. Efficiency was defined as the relative reduction in the percentage of immature sperm compared to that of fresh semen. We noted treatment by density gradient centrifugation (ICSI-DGC) resulted in a 40% reduction in immature sperm chromatin compared to R-FS ($P < 0.01$). However, treatment with the PRED method was more efficient using both fresh semen samples (PRED-FS, 96.9%) and processed samples (PRED-DGC, 96.3%) ($P < 0.01$). No statistically significant difference was noted between PRED-FS and PRED-DGC ($P = 0.22$). Table 3 displays individual semen characteristics and outcomes of different sperm selection techniques. It corroborates that PRED

Table 1 Characteristics of the patients and collected spermatozoa

Characteristic	(<i>n</i> = 40)
Age (year)	34.45 ± 7.78
Volume (ml)	3.62 ± 1.85
Sperm concentration ($\times 10^6/\text{ml}$)	62.18 ± 53.59
Progressive motility (%)	31.88 ± 17.90
Normal sperm morphology—WHO (%)	13.31 ± 8.91

Values are mean $\pm$ SD

WHO World Health Organization

Fig. 6 Dyes were used to improve visualisation of the fluid dynamics in the sperm selection approach. **a** A PRED circuit was assembled on a standard ICSI dish. Red dye was added to the PVP solution. One drop of 10 μ L of culture medium stained in yellow was added to the pressure control reservoir (PCR). Another drop of 10 μ L containing blue-coloured culture medium was added to the seminal deposition reservoir (SIR), mimicking a semen sample. **b** Culture medium (yellow) starts to flow through the channel from PCR; PVP (red) diffuses slowly through PCR;

the semen sample (blue) remains confined within the SIR. The culture medium reaches the SIR and prevents the spread of the semen sample through the channel. **c** The shift from blue to green demonstrates the steady influx of culture medium into the SIR, preventing the spread of debris and contaminants; PVP gradient formation in the PCR; the spermatozoa must swim against the flow and across a PVP gradient to reach the sperm collection point

is superior to DGC in both improving morphology and reducing chromatin immaturity regardless of seminal quality.

Discussion

In this preliminary study, an effective approach based on MFSSs was designed to achieve safe selection of individual sperm in an ICSI dish without previous semen processing. We have demonstrated that 15 μ L of unprocessed semen is able to provide 50 or more sperm to be used in ICSI within 30 min for most cases using the PRED procedure, even in cases with poor semen quality. This method was more efficient in reducing sperm chromatin immaturity than DGC. The head morphology of sperm selected by our device was also significantly improved compared to DGC.

The PRED approach is essentially an extended ICSI drop [25, 26] with particular characteristics that allow for sperm selection and recovery in a single-step procedure. It consists of two culture medium reservoirs (one containing a PVP solution) connected by a channel. When a sperm sample is introduced into the inlet reservoir, spermatozoa move randomly away from the bulk and accumulate near the boundary [27], leaving debris and immotile, slower sperm behind. By swimming along the boundaries [28], motile sperm reach the junction between the inlet and the channel, where they are swept by the flow inside the channel. The flow is driven by the hydrostatic pressure created by the height of culture medium columns in the reservoirs [29]. The height in the reservoirs changes slightly as fluid flow occurs. Initially, the flow in the channel overwhelms the swimming ability of the sperm, but in this gravity-driven pumping system, the flow rate decreases over time as the volume of fluid in the outlet reservoir

Table 2 Comparison of normal morphology and chromatin immaturity in the processed groups

	R-FS	ICSI-DGC	PRED-FS	PRED-DGC
Normal morphology (%)	12.11 $\pm$ 8.93	27.91 $\pm$ 11.67 ^a	57.59 $\pm$ 9.47 ^{b,c}	56.82 $\pm$ 9.28 ^{d,e}
Abnormal chromatin condensation—AB (%)	28.65 $\pm$ 8.97	17.29 $\pm$ 7.72 ^a	0.89 $\pm$ 1.31 ^{b,c}	1.05 $\pm$ 1.63 ^{d,e}

There was no significant difference between PRED-FS and PRED-DGC regarding morphology ($P = 0.47$) and chromatin condensation ($P = 0.25$)

R-FS raw fresh semen, *ICSI-DGC* ICSI density gradient centrifugation, *PRED-FS* PRED fresh semen, *PRED-DGC* PRED density gradient centrifugation, *AB* aniline blue

^a Significant difference between ICSI-DGC and R-FS sperm fractions ($P < 0.001$)

^b Significant difference between PRED-FS and R-FS sperm fractions ($P < 0.001$)

^c Significant difference between PRED-FS and ICSI-DGC sperm fractions ($P < 0.001$)

^d Significant difference between PRED-DGC and R-FS sperm fractions ($P < 0.001$)

^e Significant difference between PRED-DGC and ICSI-DGC sperm fractions ($P < 0.001$)

Fig. 7 Relative reduction in aniline blue staining (chromatin immaturity) compared to raw fresh semen (R-FS). ICSI-DGC ICSI density gradient centrifugation, PRED-FS PRED fresh semen, PRED-DGC PRED density gradient centrifugation. Box plots: the bottom and top of the box represents the first and third quartiles, the band inside the box refers to the median and the ends of the whiskers represent the minimum and the maximum scores

decreases [30], thereby allowing highly motile spermatozoa to swim against the flow—positive rheotaxis [13]. Sperm cells exhibiting positive rheotaxis behaviour align against the flow and spread through the channel [31, 32], reducing the likelihood of a sperm-sperm collision [28], and thus increasing the area available to the swimmers. The highly motile spermatozoa swim against the flow through the long channel (~30 mm) to reach the PCR. In this sector, the gametes must penetrate a viscosity gradient until they are recovered by an ICSI micropipette.

The PRED approach shares similarities with the swim-up method but incorporates some innovative characteristics from microfluidic sperm selection devices, such as long, space-constrained channels [33], gravity pump-driven flow [29] and high-viscosity fluids [17]. To our knowledge, this is the first time all of these features have been used in the same approach, not as a microfluidic device, but as a modified ICSI drop.

One major criticism of the ICSI technique has been that visual inspection alone can lead to the inadvertent selection of sperm with various levels of pathology [5, 34, 35]. Among currently available semen processing methods, DGC is the most commonly used preparation technique in assisted reproductive technology (ART) laboratories [36, 37]. This technique has been proven to be efficient for selecting motile and morphologically normal sperm but tends to generate reactive oxygen species (ROS) during the centrifugation steps, which have been shown to potentially impair sperm function [38]. Recent studies have questioned the safety of this procedure, suggesting the induction of de novo DNA damage [39,

40]. Our results have demonstrated that PRED-FS was able to provide sperm with better maturity and morphology in comparison to ICSI-DGC and PRED-DGC. These findings have not been described before but are in accordance with the above-mentioned studies that reported negative effects of centrifugation on sperm quality.

New microfluidic sorting systems allow for the selection of sperm with better morphology, improved chromatin maturity and higher DNA integrity than sperm selected by traditional methods [17, 19, 20]. One of the merits of using a microfluidic device in ART is its one-step sorting protocol, without the need for centrifugation, which minimises the exposure of sperm to ROS and prevents DNA fragmentation [41].

By using a method based on microfluidic devices, we obtained comparable results for improving sperm morphology and chromatin maturity status. Improvements in DNA integrity might also occur because our approach shares the same selection features as microfluidic devices. It has been demonstrated that there is a correlation between poor protamination and DNA fragmentation [42]. Since PRED was able to reduce substantially the percentage of sperm containing immature chromatin, it would be reasonable to infer that the rates of DNA fragmentation might have also been reduced.

The PRED method may improve the current ICSI technique by adding its own sperm selection process, no longer depending on seminal processing techniques. It would reduce the dependence on andrology laboratories and could also be beneficial for small laboratories that have only one embryologist. ICSI would probably become an even more complete technique comprising selection, capture and injection of the male gamete. In addition, this method of sperm selection is rapid and easy to use; no additional qualified technicians are required and the same ICSI dish is used. No specific training for embryologists is required and reproducibility should be of little concern. These features might eventually reduce the costs and time of ICSI procedures.

One of the limitations in the present study is that DNA integrity analysis was not performed. Unfortunately, we were not able to perform such analysis with the resources available at the time of the study. Moreover, processing of semen samples with very high viscosity, very low sperm count (<500,000/ml) or poor motility (<1%) might encounter technical difficulties using the PRED approach. In these cases, we recommend washing the seminal sample before using the PRED method. Even if there is a concentration of dead cells and debris, the gametes with good motility will migrate to the edges and swim towards the main channel as shown in Supplementary video 4. This case was only a test and is not part of the main study. As a way of reducing processing time and to allow the use of poor seminal samples, after sperm washing, shorter versions of the circuit are being tested in our laboratory.

Table 3 Comparison of normal morphology and chromatin immaturity individually

ID	Age (year)	Vol (ml)	Count $\times 10^6$	Motil. Prog. (%)	Morph-FS (%)	Morph-DGC (%)	Morph-PRED-FS (%)	Morph-PRED-DGC (%)	AB-FS (%)	AB-DGC (%)	AB-PRED-FS (%)	AB-PRED-DGC (%)
5	39	4.5	6	26	1.50	7.50	56.96	54.79	50.00	32.00	6.33	7.53
27	20	1.5	49	63	1.50	11.00	51.55	54.87	43.50	24.50	3.11	4.10
17	36	2.6	10	26	2.50	11.50	56.14	56.52	46.00	32.50	5.26	6.52
1	34	3.5	8.2	11	3.00	13.00	52.00	50.75	31.00	27.00	1.33	0.75
6	39	2.8	30	19	3.00	15.50	48.55	50.88	28.50	17.50	0.00	0.00
14	27	3	7	20	3.00	17.00	48.26	55.77	27.50	16.00	0.00	0.00
24	20	1.7	40	35	3.00	7.50	42.31	37.96	34.50	28.50	0.00	0.93
33	41	2.8	42	19	3.00	18.00	48.51	50.96	32.00	15.00	0.50	0.48
39	26	1.7	35	43	4.00	17.50	52.49	58.03	34.50	18.50	0.55	0.52
8	36	2	16	8	4.50	28.00	61.19	56.86	34.00	19.00	0.46	0.49
19	41	3.2	9	35	4.50	28.00	64.42	59.80	34.00	19.00	0.00	0.00
38	32	2.5	65	14	5.50	30.00	58.25	58.37	28.50	10.50	0.49	0.48
15	20	2	9	58	6.50	21.00	43.27	33.94	31.00	24.00	0.96	1.38
25	31	2.2	32	21	6.50	21.00	39.47	33.33	31.00	24.00	0.88	1.43
34	43	5.5	52	16	7.00	37.50	43.94	46.12	43.00	28.00	0.51	0.49
7	41	5.5	72	6	7.50	28.50	43.37	45.02	45.50	38.00	0.51	0.47
30	47	7	56	7	7.50	25.50	56.63	53.77	37.00	15.50	1.20	0.94
13	36	4.5	3.5	7	8.50	29.50	56.19	60.98	25.00	9.00	0.95	0.49
21	29	2.5	76	14	8.50	29.50	60.26	60.27	25.00	9.00	0.44	0.46
28	40	2.3	194	15	9.00	16.50	50.96	50.92	23.50	17.50	0.00	0.92
36	35	2.6	15	26	11.00	24.24	63.69	59.38	41.00	28.28	1.79	3.13
12	26	2.8	75	59	12.00	39.50	65.50	62.70	21.00	16.50	0.87	1.23
16	37	2	30	7	12.50	18.50	45.76	45.67	21.00	17.50	1.69	1.57
20	48	7	54	16	12.50	31.50	58.42	56.86	18.00	14.00	0.99	0.49
4	39	3.8	66	32	13.00	39.50	69.82	66.83	20.00	10.50	0.45	0.48
32	36	3.8	54	32	13.00	39.50	77.11	67.48	20.00	10.50	0.00	0.00
22	40	4.5	8	13	13.50	25.50	51.13	53.02	30.50	19.50	0.75	1.34
37	39	2.5	35	28	14.50	27.00	65.52	64.36	17.00	10.00	0.49	0.00
9	46	8.2	85	50	16.00	28.50	64.92	64.64	31.00	15.00	1.05	1.10
23	34	4	160	56	16.00	31.50	69.20	66.22	21.50	16.00	0.45	0.44
18	38	2.5	37	22	17.00	25.00	68.04	72.19	14.50	12.00	0.46	0.00
26	35	4	160	28	19.50	37.00	52.44	49.72	25.00	12.50	0.89	1.66
3	33	2.5	156	20	20.00	31.00	58.88	63.24	20.00	11.00	0.00	0.00
31	37	2.7	86	20	21.57	31.34	58.42	62.00	21.57	11.44	0.00	0.00
40	34	4.1	88	31	25.74	40.00	60.00	58.71	24.26	12.50	0.00	0.00
10	33	2	74	55	26.50	41.50	72.12	71.50	18.00	10.00	1.44	1.40
29	30	5.2	102	32	26.50	39.00	58.91	58.49	23.50	13.50	0.00	0.00
2	20	1.5	36	55	27.50	40.00	65.93	67.79	33.00	14.00	0.00	0.00
35	29	4	61	54	31.50	56.00	71.29	64.42	20.50	6.50	0.50	0.48
11	22	4	74	61	34.50	57.00	71.71	67.83	19.50	5.50	0.49	0.43

Morphology WHO (2010)

PRED positive rheotaxis extended drop, *DGC* density gradient centrifugation, *Motil-Prog* progressive motility, *Morph-FS* morphology fresh semen, *Morph-DGC* morphology after DGC, *Morph-PRED-FS* morphology after PRED-fresh semen, *M-PRED-DGC* morphology after PRED-DGC, *AB-FS* aniline blue fresh semen, *AB-DGC* aniline blue after DGC, *AB PRED-FS* aniline blue after PRED fresh semen, *AB PRED-DGC* aniline blue after PRED-DGC

Conclusion

In conclusion, a novel one-step ICSI approach combining sperm selection and recovery was developed and a preliminary test was performed. This PRED approach can easily select sperm and enable the direct use of native semen in ICSI. It can select sperm with better morphology and chromatin maturity than the DGC method regardless of sperm concentration. Further studies are needed to assess its ability to reduce sperm DNA fragmentation and its role in clinical practice.

DGC, density gradient centrifugation; FS, fresh semen; ICSI, intracytoplasmic sperm injection; MFSS, microfluidic sperm sorter; PCR, pressure control reservoir; PRED, positive rheotaxis extended drop; PVP, polyvinylpyrrolidone; SIR, semen input reservoir

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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