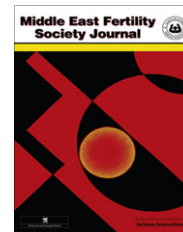




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ORIGINAL ARTICLE

# Pregnancy outcome after IUI for male and idiopathic infertility using a new simplified method for sperm preparation<sup>☆</sup>

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## KEYWORDS

IUI;  
Ovarian stimulation;  
Sperm preparation;  
Swim-up

**Abstract** *Introduction:* Intrauterine insemination (IUI) is a valid treatment for infertility with a cumulative pregnancy rate of 40–90% after 3–10 treatment cycles.

*Design:* We prospectively studied the efficacy of a new simplified method for motile sperm preparation for IUI for both male causes of infertility, and for those diagnosed with idiopathic infertility.

*Methods:* A prospective clinical trial was performed with 200 couples, with a 2–8 years history of primary infertility. One hundred couples had been diagnosed as idiopathic, while another 100 couples with male factors of infertility. Motile sperm for IUI was prepared by: (A) the classic World Health Organization self-migration (swim-up) method which includes centrifugation, or (B) the proposed simplified swim-up procedure without centrifugation. Both anti-estrogens and HMG had been used for ovarian stimulation. Depending on the cause of infertility, patients were matched one-to-one at the time of IUI, so that when a total of 100 couples had been treated of both causes of infertility, 50/100 women received sperm prepared by method A and 50/100 by method B.

*Results:* A statistically significant correlation was found between the percentage motile sperm of the original semen sample and the percentage of motile sperm recovered by method A ( $r = 0.333$ ,  $P < 0.01$ ) and B ( $r = 0.400$ ,  $P < 0.01$ ). A highly significant correlation ( $r = 0.997$ ,  $P < 0.001$ )

<sup>☆</sup> The research work had been done in El-Minya University Hospital, a tertiary referral center in upper Egypt.

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was found between the two methods. The pregnancy outcome after the proposed IUI procedure was not significantly different after the traditional swim-up sperm preparation.

**Conclusions:** The simple proposed swim-up method was as effective as the classic swim-up method, regarding the sperm recovery and pregnancy outcome, but in an easier, and faster way.

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## 1. Introduction

Intra uterine insemination (IUI), together with ovarian stimulation, is a less expensive and invasive treatment in comparison with other assisted reproductive technique (ART) (1), and has been widely used for the treatment of infertile couples with a variety of indications, such as non severe male factor infertility, unexplained infertility, cervical mucus hostility and ovulatory disturbances (2). Ovarian stimulation with exogenous gonadotrophins, combined with intrauterine insemination (IUI), is a valid treatment for infertility. Its effectiveness in terms of pregnancy rate is ~10–14% per cycle reaching cumulative values of 40–90% after 3–10 treatment cycles (1,2).

Variance in pregnancy rate achieved may be due to the small size of the study populations, variability in characteristics of subjects and ovarian stimulation and treatment protocols, including maternal age (3–6), timing and frequency of insemination (7,8), number of treatment cycles, use of ovarian stimulation regime (9), etiology and duration of infertility and total number of motile sperm inseminated (10). Although results may appear concordant for some of these factors, a lack of consistency is still evident for some others, such as female age, size of follicles and ovulatory side (2).

One of the main premises for these results is the preparation of morphologically normal, motile sperm. Since the human ejaculate consists of a heterogeneous mixture of sperm with variable motility, sometimes agglutinated or malformed, together with erythrocytes, leukocytes, germinal cells, epithelial cells and amorphous material, it is necessary to prepare a sperm sample for IUI that contains mainly sperm of normal conformation and progressive rectilinear motility (2,6,7).

In line with current World Health Organization recommendations, the preferred method for sperm preparation in our laboratory is the swim-up method (World Health Organization, 1999). This system is also used for oligo or asthenozoospermic samples, when an adequate recovery of motile sperm is documented during the diagnostic phase (8–10).

In order to recover these sperms, various techniques, most of which involve separation of cells from the liquid phase by centrifugation, are used. However, centrifugation may damage sperm and increasing the number of technical steps increases the possibility of sample contamination and operator contact with biological material (11–13). The aim of the current study is to evaluate a new proposed simplified technique for sperm preparation for IUI for both male factors of infertility versus for those diagnosed as idiopathic or un-explained infertility.

## 2. Materials and methods

### 2.1. Study design

This is a prospective controlled study, where 200 couples were included, 100 with male factors of infertility, and another 100

couples were diagnosed as idiopathic infertility, with female age between 20 and 35 years old, male age between 30 and 45 years old, and with a 2–8 year history of primary infertility. Hysterosalpingograms (HSG) were normal. It is a double armed study: the first arm has studied the newly innovated sperm preparation compared to the old standardized one, and the second arm has studied the final pregnancy outcome after using this new simplified procedure in male and un-explained infertility cases.

Oligozoospermia was classified as  $5\text{--}20 \times 10^6$  sperm/ml with normal motility and morphology and asthenozoospermia was classified as 20–50% motility (type a + b) with normal count and morphology. Azospermic men, and those with seminal analysis less than 5 million/mL had been excluded from the study. Depending on the cause of infertility, the patients were matched one-to-one at the time of IUI, so that when a total of 100 couples for both groups of infertility had been treated, 50/100 women received sperm prepared by method A, 50/100 sperm prepared by method B and the causes of infertility were equally distributed between the two groups.

Ovarian stimulation began on day 2 of the menstrual cycle, using Clomiphene citrate with 50–100 mg/day for 5 days according to the women's body weight. Transvaginal ultrasonography had been used for follicular monitoring, and to exclude transonic formations > 10 mm in diameter. HMG (Merional, IBSA, Egypt) was administered at a starting dose of 75–150 IU/day, increased to a maximum of 300 IU/day if necessary according the ovarian response, until optimum conditions for administration of HCG were reached, till reaching at least one dominant follicle between 18 and 20 mm in two diameters.

Ultrasound examination was performed with a 5/6/7.5 MHz vaginal probe (Sonoline SL-1, Medison AG, Korea) on day 8 of ovarian stimulation. Follicle size was recorded as the mean of two perpendicular diameters for each follicle. Endometrial thickness was measured along the longitudinal axis of the uterus. At each examination follicular number, size and endometrial thickness were recorded.

At examination on day 8, the dose of HMG was maintained or modified, depending on the presence or otherwise of one or more follicles measuring > 12 mm in diameter. Ultrasound monitoring was performed on day 11, and the dose was adjusted until at least one follicle 18–20 mm in diameter. At this stage, (10000 IU HCG-Choriomon: IBSA, Egypt) was administered.

### 2.2. Sperm processing

Specimens of seminal fluid were obtained by masturbation and collected in sterile containers at the Assisted Procreation Unit. Morphology was evaluated in pre-stained slides (Testsimplets: Boehringer, Germany) using the criteria of Kruger (14,15). Sperm count and motility were assessed by World Health Organization criteria, by placing 0.5 mL of semen on to a Makler chamber, under laminar flow and observing by light

microscope (Nikon Instruments, Korea). Original sperm specimens and two samples prepared as described below were assessed.

Preparation and assessment were performed by a single experienced operator. Accuracy and precision are routinely checked in our laboratory by analysis of 10 repeated preparations of a representative masked sample (intra-technician variation).

The coefficient of variation (CV) for sperm count was 8.1 and 8.6%, and for motility (type a + b) 9.0 and 8.8% at the beginning and end of the study. The same Makler chamber was employed for all samples examined throughout the study. Accuracy was assessed by comparison with haemocytometer: 10 different samples were masked and evaluated by the two methods at the beginning and end of the study. The sperm preparation medium (buffered Earl's Balanced Salt Solution (HEBES) + 0.4% HSA: Medi-Cult Universal, Jyllinge, Denmark) used for sperm preparation was kept at 37 °C for 1 h before use.

### 2.3. Method A (Fig. 1)

Using a sterile pipette, 1 ml semen was placed in a conical tube and 1 ml culture medium was slowly layered on top. The tube was sealed, inclined at 45° and stored at 37 °C for 60 min. A sterile Pasteur pipette was used to remove the supernatant containing sperm that had swum-up.

This supernatant was transferred to a sterile conical tube to which 2 ml culture medium was added and centrifuged at 300 g for 10 min. The supernatant was discarded by slow aspiration with a Pasteur pipette and the pellet resuspended in 1 ml medium which was stored at 37 °C until use.

### 2.4. Method B (Fig. 1)

Culture medium (1 ml) HEPES-buffered EBSS + 0.4% HSA, (Medi-Cult Universal), and 1 ml sperm were in turn aspirated with a 5 ml syringe to create a double layer. Aspiration was begun slowly with the syringe in vertical position with the piston upward.

The syringe was then sealed with a sterile cap, inclined at 45° and stored at 37 °C for 60 min. The seminal fluid under the medium was then ejected dropwise up to a volume equal to that originally aspirated. The remaining volume (1 ml), containing motile sperm, was stored at 37 °C until use.

### 2.5. Insemination technique

IUI was performed 36 h after administration of HCG. The cervix was wiped with a few ml of medium and the catheter (Tomcat, Sherwood, MO, USA) was gently introduced into the uterus until it touched the fundus. It was then retracted ~1 cm and the sperm injected with a slow movement of the piston. The patient remained supine for ~10 min and then resumed normal activity.

Transvaginal micronized progesterone was prescribed 400 mg/day, (Prontogest, IBSA, Egypt) starting on the day of insemination or the next day and continuing until the next menstrual period and/or diagnosis of pregnancy (intrauterine gestation sac with evidence of an embryonic heartbeat).

### 2.6. Statistical analysis

All data distributions were tested for skewness and kurtosis using the Statistics Package for Social Sciences software package version (SPSS Inc., Chicago, IL, USA). One sperm parameter (sperm count) had asymmetric distribution and was analyzed by non-parametric methods (Spearman's rank correlation coefficient and Mann-Whitney *U*-test). The other variables showed normal distributions and were analyzed by Pearson's correlation coefficient and Student's *t*-test. The significance level was set at  $P < 0.05$ .

## 3. Results

Tables 1 and 2 show the results of the two methods in terms of number and motility, before (basal sperm analysis) and after swim-up, performed either by method A or B for both causes of infertility included. Ovarian stimulation characteristics are reported in the same table: number of follicles > 18 mm in

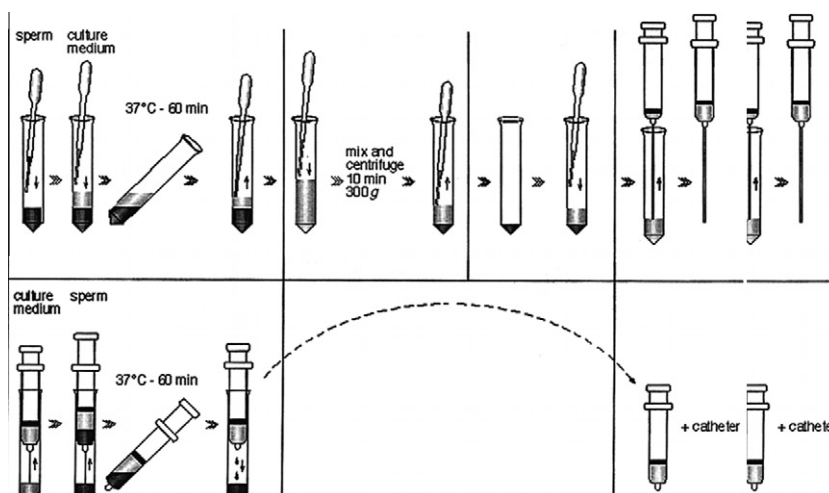
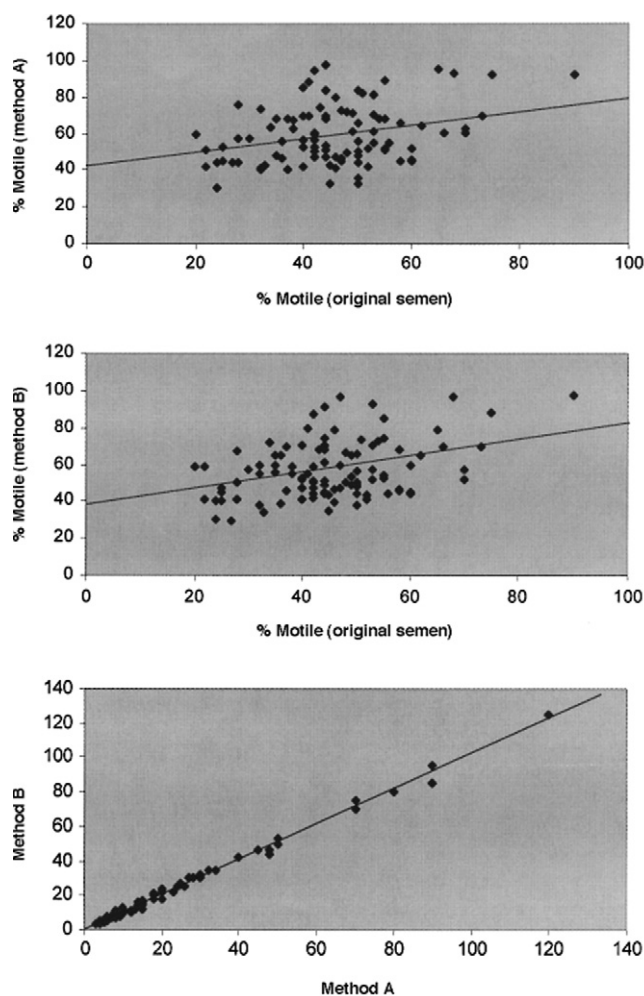


Figure 1 Motile sperm preparation: upper method (A), lower method (B).



**Figure 2** Correlation between motile sperm in the original semen sample and swim-up samples obtained by method (A) top, or (B) middle and between the two methods (bottom) in idiopathic cases of infertility.

diameter ranged from one to three at the time of HCG administration, length of stimulation was 10–16 days, total number of HMG units used per cycle was 600–1200 IU, endometrial thickness was 9–14 mm at the time of HCG administration.

A statistically significant correlation was found between sperm number  $\times 10^6$  in semen and swim-up samples ( $P < 0.001$ , Spearman's rank correlation coefficient) prepared by method A ( $r = 0.874$ ) and method B ( $r = 0.874$ ). A linear correlation was found between percentage of motile sperm

(type a + b) (World Health Organization, 1999) in the original specimen sample and percentage of motile sperm (type a) recovered by method A (Fig. 2, upper,  $r = 0.333$ ,  $P < 0.01$ ) and method B (Fig. 2, middle,  $r = 0.400$ ,  $P < 0.01$ ), respectively. Similar significance was found comparing the number of motile sperms recovered with the two methods (Fig. 2, lower,  $r = 0.997$ ,  $P < 0.001$ ).

Our results showed that infertility duration less than 5 years is associated with a significantly better pregnancy rate compared with a longer duration of infertility (27.3% vs. 12.2%, respectively). In addition, most pregnancies (84.4%) were obtained within the first two treatment cycles. All of the pregnancies occurred within the first four treatment cycles and no pregnancy was achieved in the fifth, sixth and seventh cycles. The actual number of pregnancies and the percentage pregnancy rate are reported for groups A and B for both causes of infertility included (Tables 1 and 2). We found an overall multiple pregnancy rates of 3.9% in our study. Multiple gestations seem to be much less frequent after IUI compared with that generally reported in IVF and intracytoplasmic sperm injection (ICSI) (25–30%).

No statistically significant differences were found between the two groups in these parameters. There were no differences in the number of stimulated follicles between pregnant ( $1.66 \pm 0.21$  mm) and non-pregnant ( $1.4 \pm 0.06$  mm) women. There was a slightly better sperm number recovered after the proposed sperm preparation method than the classic swim-up procedure is especially for male cases of infertility, which is actually not statistically significant. The pregnancy outcome was as well slightly and insignificantly higher after the proposed simplified method of sperm preparation. Figs. 2 and 3

Logistic regression analysis revealed four predictive variables as regards pregnancy: the total sperm numbers, number of the treatment cycle (OR: 3.5 CI: 1.9–6.4,  $P = 0.006$ ), duration of infertility (OR: 2.1 CI: 1.2–3.7  $P = 0.001$ ) and age (OR: 2.15, CI: 1.1–4.4,  $P = 0.04$ ), with a slightly better outcome after the proposed simple method of sperm preparation, especially for those with idiopathic infertility. In other words, best pregnancy rates were obtained in patients with younger age and fewer cycles and years of infertility complaints. Pregnancy rate did not have any independent relation to the number of preovulatory follicles.

#### 4. Discussion

The present results show that the simplified swim-up method for recovery of motile sperm is reliable. One advantage of the method is the limited number of technical steps and the

**Table 1** Pregnancy outcome for idiopathic infertility cases.

Basal sperm		Recovered sperm		Follicle no. (> 18 mm)	Stimulation length (days)	Total HMG (IU)	Endometrial thickness (mm)	Pregnancy rate
Conc. (10 <sup>6</sup> ml <sup>-1</sup> ) <sup>b</sup>	Motility (%)	Conc. (10 <sup>6</sup> ml <sup>-1</sup> ) <sup>b</sup>	Motility (%)					
Method A <sup>a</sup> (n = 50)								
45.0 (28–70)	46.7 ± 1.67	13.5 (8–25)	93.5 ± 4.5	1.4 ± 0.1	11.7 ± 0.20	832 ± 18.7	11 ± 0.16	6/50 (12.0%)
Method B <sup>a</sup> (n = 50)								
41.0 (25–60)	43.98 ± 1.92	11.0 (8–22)	95.1 ± 3.3	1.5 ± 0.08	11.9 ± 0.31	850 ± 21.3	10.7 ± 0.19	7/50 (14.0%)

n is the number of patients.

<sup>a</sup> See Section 2.

<sup>b</sup> Median and interquartile range.



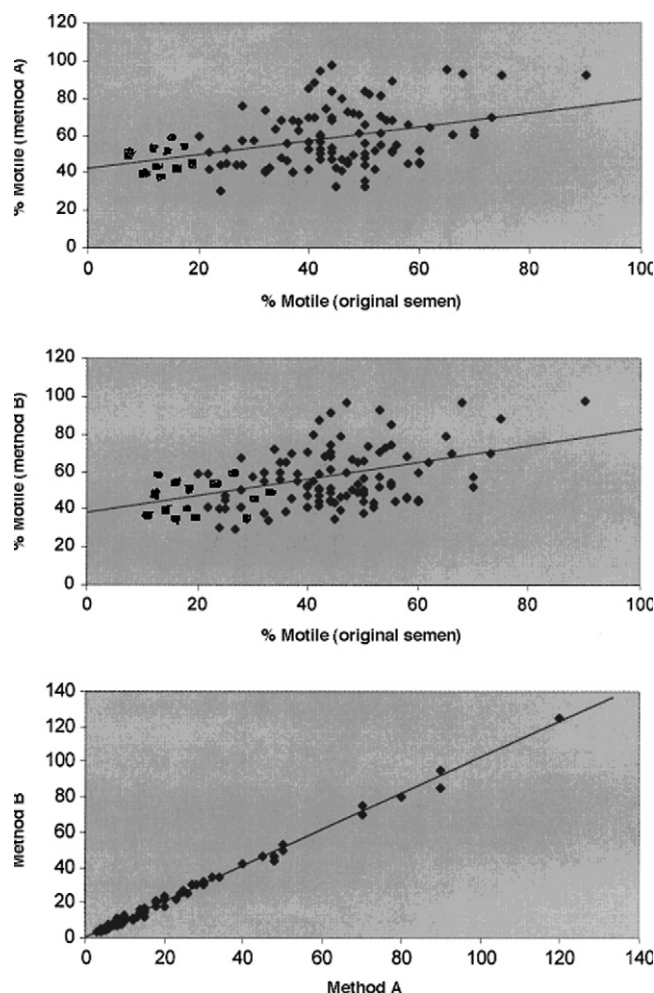
**Table 2** Pregnancy outcome for male infertility cases.

Basal sperm		Recovered sperm		Follicle no. (> 18 mm)	Stimulation length (days)	Total HMG (IU)	Endometrial thickness (mm)	Pregnancy rate
Conc. (10 <sup>6</sup> ml <sup>-1</sup> ) <sup>b</sup>	Motility (%)	Conc. (10 <sup>6</sup> ml <sup>-1</sup> ) <sup>b</sup>	Motility (%)					
Method A <sup>a</sup> (n = 50)								
15.0 (9–20)	15.7 ± 1.67	5.5 (4–8)	73.5 ± 4.5	1.1 ± 0.1	12.7 ± 0.20	822 ± 18.7	12 ± 0.16	3/50 (6.0%)
Method B <sup>a</sup> (n = 50)								
12.0 (8–18)	12.98 ± 1.92	4.5 (3–7)	75.1 ± 3.3	1.2 ± 0.08	12.9 ± 0.31	841 ± 213	11.7 ± 0.19	4/50 (8.0%)

*n* is the number of patients.

<sup>a</sup> See Section 2.

<sup>b</sup> Median and interquartile range.



**Figure 3** Correlation between motile sperm in the original semen sample and swim-up samples obtained by method (A) top, or (B) middle and between the two methods (bottom) in male cases of infertility.

lack of a centrifugation step, which besides being more practical, avoids a procedure which could potentially damage the spermatozoal cytoplasmic membrane (15,16). The aim of various research projects has been to simplify sperm preparation. Besides the traditional methods of swim-up and centrifugation on a discontinuous density gradient, various other techniques have been developed. These include swim-up with antigravita-

tional centrifugation, 'multi-ZSC' column for 'in office' preparation of human sperm, or migration into a higher density culture medium, such as swim-down (17–19).

These and other techniques are designed to recover morphologically better motile sperm. Some procedures are preferred because of their capacity to maximize motile sperm recovery. Sperm recovery does not necessarily need to be maximized, but is adequate for a good pregnancy rate per cycle. According to a recent paper, the lower limit for a good pregnancy rate after IUI is a motile sperm concentration of  $5 \times 10^6$  ml $^{-1}$  in the original sperm sample and a total count of  $10 \times 10^6$  and 30% progressive motility, or alternatively a total motile sperm count of  $5 \times 10^6$ . The authors indicate  $1.6 \times 10^6$  motile sperm as a limit below which pregnancies do not occur (20,21).

Previous papers showed that a total motile sperm count after swim-up of  $0.8 \times 10^6$  was associated with a good pregnancy rate, which did not increase if more sperms were introduced. These studies indicate that a good pregnancy rate can be obtained with relatively low motile sperm counts and well-timed insemination and is more effective than a high sperm count (20–24).

The percentage sperm recovery obtained in the present study ensures efficacy of the method, indeed, the pregnancy rates obtained in the present study were similar to those reported in the literature. The one-step swim-up method can probably be used with oligoasthenozoospermic samples having predicted sperm recovery above the reported limits. In cases of severe oligozoospermia, other methods, involving centrifugation and concentration of sperm, seem advisable (25–27).

In agreement with the results published by Sinikka et al., and in contrast to Kang and Wu who described a slight though statistically significant higher pregnancy rate in relation to the number of motile sperms inseminated, in our study there was no evidence of a significant correlation between number of sperms and pregnancy rate. Others have also failed to find differences. This is obviously due to pre-treatment sperm screening and exclusion of couples with a progressively motile sperm count after preparation of  $6 < 1 \times 10^6$  ml $^{-1}$ .

The simplified method of sperm preparation does not require particular expertise and saves material, it is, therefore, more practical for the operator and less expensive. Furthermore, the reduction of technical steps enables sperm to be prepared in the office rather than in a specialized laboratory. Finally, the simplified method is practically a closed system as the procedure takes place inside a syringe, with the double advantage of safety for the operator against biological material that could be a source of disease, and protection of the sample

from contamination. Regarding the policies and procedures of motile sperm preparation, the present technique reduces the risks without being a detriment to the results (28–30).

New updated studies have confirmed the presence of many problems developed after the standardized swim-up sperm preparation. In addition to the greatly possible contamination and leukocytospermia developed after the multiple steps of the standardized procedure, the studies confirmed the generation of several oxygen species and mitochondrial membrane potential after the centrifugation step. A recent flow cytometric study, and another high magnification microscopy study had documented the newly developed morphological changes which negatively affect the sperm integrity (31–34).

In conclusion, the simplified method of sperm preparation for IUI is a step toward optimization of the procedure and reduction of time and costs, enabling the gynecologist to work in a safe and effective way. The proposed sperm preparation procedure is a good opportunity for small gynecology units which lack the highly sophisticated instruments to help infertile couples. The current work is in need for further researches, studying effects of the proposed and classic swim-up procedures on the ultra-structure of the sperm parameters, using different detailed imaging, biochemical and genetic sperm parameters, which might affect to a great extent the establishment of the current proposed sperm preparation procedure.

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