Reduction of steps in the preparation of motile sperm for intrauterine insemination does not reduce efficacy of the procedure: simplified one-step swim-up method versus classic swim-up

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BACKGROUND: Intrauterine insemination (IUI) is a valid treatment for infertility with a cumulative pregnancy rate of >40–90% after 3–10 treatment cycles. We studied the efficacy of a simplified method for motile sperm preparation for IUI. METHODS: A prospective clinical trial was performed with 100 couples (male age 33–48 and female 28–37 years) with a 2–8 year history of primary infertility associated with slight oligozoospermia (16/100), oligomenorrhoea (32/100) or unknown (52/100). Motile sperm for IUI were prepared by: (A) the classic World Health Organization self-migration (swim-up) method which includes centrifugation, or (B) a simplified one-step swim-up procedure without centrifugation. Recombinant FSH was 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, 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 ($r = 0.997, P < 0.001$) was found between the two methods. CONCLUSIONS: The simplified one-step swim-up method was as effective as the classic swim-up method, but the former was easier and more economical.

Key words: IUI/ovarian stimulation/sperm preparation/swim-up

Introduction

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 (Ho et al., 1992; Karlstrom et al., 1993; Ombelet et al., 1995; Cohlen et al., 1998; Goverde et al., 2000) reaching cumulative values of >40–90% after 3–10 treatment cycles (Comhaire et al., 1994; Ombelet et al., 1995, 1997; Goverde et al., 2000). 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.

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. In order to recover these sperm, various techniques (Karabinus and Gelety, 1997; Babbo et al., 1999; Zavos et al., 2000), most of which involve separation of cells from the liquid phase by centrifugation, are used. However, centrifugation may damage sperm (Agarwal et al., 1994; Shekarriz et al., 1995; World Health Organization, 1999) and increasing the number of technical steps increases the possibility of sample contamination and operator contact with biological material. A simplified method of sperm swim-up to use for IUI is reported in the present study.

Materials and methods

Study design

The subjects were 100 couples (male 33–48 and female 28–37 years) with a 2–8 year history of primary infertility associated with oligoasthenozoospermia (16/100), oligomenorrhoea (32/100) or unknown (52/100). Hysterosalpingograms were normal. Oligozoospermia was classified as 10–20×10⁶ sperm/ml with normal motility.
and morphology and asthenozoospermia was classified as 20–50% motility (type a+b) with normal count and morphology.

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 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, after transvaginal ultrasonography to exclude transonic formations >10 mm in diameter. Recombinant (r)FSH (Gonal-F; Serono Pharma, Rome and/or Puregon; Organon, Rome, Italy) was administered at a starting dose of 50–75 IU/day, increased to a maximum of 150 IU/day if necessary, until optimum conditions for administration of HCG were reached.

Ultrasound examination was performed with a 5/6/7.5 MHz vaginal probe (Sonoline SL-1, Siemens AG, Erlangen, Germany) 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 rFSH 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 exceeded 20 mm in diameter. At this stage, 5000 IU HCG (Profasi; Serono Pharma) was administered.

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 (Testisimplets; Boehringer Mannheim, Mannheim, Germany) using the criteria of Kruger (Menkveld et al., 1990). Sperm count and motility were assessed by World Health Organization criteria (World Health Organization, 1999), by placing 5 µl of semen on to a Makler chamber (Makler, 1980), under laminar flow and observing by light microscope (Nikon Instruments, Florence, Italy) (World Health Organization, 1999). 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 (HEPES-buffered EBSS + 0.4% HSA; Medi-Cult Universal, Jyllinge, Denmark) used for sperm preparation was kept at 37°C for 1 h before use.

Method A (Figure 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.

Method B (Figure 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.

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. If more than one mature follicle was present, transvaginal micronized progesterone was prescribed 100 mg/day, (Esolut, Angelini, Rome, Italy) 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).

**Statistical analysis**

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

**Results**

A statistically significant correlation was found between sperm number \( \times 10^6 \) in semen and swim-up samples \( (P < 0.001, \text{Spearman’s rank correlation coefficient}) \) 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 (Figure 2, upper; \( r = 0.333, \ P < 0.01 \)) and method B (Figure 2, middle; \( r = 0.400, \ P < 0.01 \)) respectively. Similar significance was found comparing the number of motile sperm recovered with the two methods (Figure 2, lower; \( r = 0.997 \; P < 0.001 \)).

Table I shows 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. Ovarian stimulation characteristics are reported in the same table: number of follicles \( > 18 \) mm in diameter ranged from one to three at the time of HCG administration; length of stimulation was 10–16 days; total number of rFSH units used per cycle was 600–1200 IU; endometrial thickness was 9–14 mm at the time of HCG administration. The actual number of pregnancies and the percentage pregnancy rate are reported for groups A and B. No statistically significant differences were found between the two groups in these parameters. Indeed, there were no differences in the number of stimulated follicles between pregnant \( (1.66 \pm 0.21 \text{ mm}) \) and non-pregnant \( (1.4 \pm 0.06 \text{ mm}) \) women.

**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 lack of centrifuge step, which besides being more practical, avoids a procedure which could potentially damage the spermatozoal cytoplasmic membrane (Agarwal et al., 1994; Shekarriz et al., 1995).

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 antigavitational centrifugation (Babbo et al., 1999), ‘multi-ZSC’ column for ‘in office’ preparation of human sperm (Zavos et al., 2000), or migration into a higher density culture medium, such as swim-down (Ing et al., 1991). 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 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 \( 5 \times 10^6 / \text{ml} \) 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 \( 5 \times 10^6 \)
One-step swim-up for motile sperm preparation

| Table I. Sperm characteristics (before and after swim-up), ovarian stimulation and pregnancy rate. Data are expressed as mean ± SEM |
|---|---|---|---|---|---|---|
| Basal sperm | Recovered sperm |
| conc. (×10^6/ml)^b | motility (%) | conc. (×10^6/ml)^b | motility (%) | Follicle no. (>18 mm) | Stimulation length (days) | Total RFSH (IU) | Endometrial thickness (mm) | Pregnancy rate |
| Method A^a |
| 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^a |
| 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%) |

^aSee Materials and methods.
^bMedian and interquartile range.

(Dickey et al., 1999). The authors indicate 1.6×10^6 motile sperm as a limit below which pregnancies do not occur. Previous papers showed that a total motile sperm count after swim-up of 0.8×10^6 was associated with a good pregnancy rate, which did not increase if more sperm were introduced (Berge et al., 1997). These studies indicate that a good pregnancy rate can be obtained with relatively low motile sperm counts and well-timed insemination is more effective than a high sperm count (Goverde et al., 2000).

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 (Goverde et al., 2000). 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 (Centola, 1997; Karabinus and Gelety 1997).

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 (Zavos et al., 2000).

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 (Gianaroli et al., 2000). Regarding the policies and procedures of motile sperm preparation, the present technique reduces the risks without detriment to the results.

In conclusion, the simplified method of sperm preparation for IUI is a step towards optimization of the procedure and reduction of costs, enabling the gynaecologist to work in a safe and effective way.

References


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