Methods of Semen Preparation for Intrauterine Insemination and Subsequent Pregnancy Rates

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ZAVOS, P.M. and CENTOLA, G.M. Methods of Semen Preparation for Intrauterine Insemination and Subsequent Pregnancy Rates. Tohoku J. Exp. Med., 1992, 168 (4), 583-590 —— Semen for insemination, either intrauterine or in vitro, must be prepared to remove seminal plasma products and/or select the healthier population of sperm prior to use. Traditionally, a double wash technique is performed, with or without subsequent swim-up to isolate the motile fraction if necessary. More recently, the use of the SpermPrep filtration method has gained acceptance, with the benefits of removal of leukocytes and seminal debris from the specimen as well as enhancement of overall sperm quality. In the current study we compared the traditional double wash method without the swim-up to SpermPrep filtration. Intrauterine inseminations (IUI's) were performed in 307 cycles on 148 infertile couples at two different infertility centers in the USA. After complete diagnostic evaluation the couples were offered IUI before proceeding to any other form of assisted reproductive technologies. Semen samples were prepared in human tubal fluid media supplemented with 5% human serum albumin (HSA; location 1) or in Ham's F-10 media supplemented with 3% HSA (location 2), either with the SpermPrep filtration method (ZBL, Inc., Lexington, KY 40523, USA) or the double sperm wash (SW) procedure. Similar sperm numbers were used for the IUI procedure in both treatment groups and locations. The SpermPrep method resulted in significantly higher pregnancy rates (PR) than the SW procedure, independent of location. The clinical pregnancy rates per cycle were statistically lower (p <0.05) in the SW group (20-22% vs. 9-10%). Of significant clinical importance, almost twice as many cycles were required in the SW group to achieve these pregnancies when compared to the SpermPrep group of patients. The results point out that IUI has a significant role to play in the treatment of many infertile couples. Furthermore, the results point out very clearly that selecting high quality spermatozoa via the SpermPrep method resulted in even higher conception rates than the SW method when IUI was applied. Similar improvements in PR using the SpermPrep method may be realized with other

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Improvements in rates of conception could be realized if spermatozoa are selected on the basis of their motility, progressive motility, and morphological characteristics. Such selection of spermatozoa could be properly applied at the time of intrauterine insemination (IUI) or other forms of assisted reproductive techniques (ART) because the seminal plasma and other background materials and debris should be removed from the spermatozoa before these procedures are performed.

A number of manipulative techniques for fresh semen are currently available to remove the undesirable spermatozoa, debris and other factors and to increase sperm quality. These various techniques include the most popular ones, simple sperm wash, swim-up or sperm rise methods (Centola and Zavos 1991), and swimdown or sedimentation type methods (Dmowski et al. 1979). Less popular methods include Ficoll centrifugation (Free et al. 1991), Percoll density gradient (Kaneko et al. 1980; Horvath et al. 1989), and Sephadex or glass wool fiber filtration (Katayama et al. 1989; McClure et al. 1989). It should be emphasized, however, that with many of these manipulative techniques the increase in sperm quality is often achieved at the expense of numbers of recovered spermatozoa that may not be especially advantageous for patients with various seminal deficiencies such as oligozoospermia. Also, equally important, the relatively lengthy time period required to perform these procedures is additionally disadvantageous because the life expectancy of low quality spermatozoa may be limited.

Recently, a new technique, SpermPrep (ZBL, Inc., P.O. Box 23777, Lexington, KY 40523, USA), has been introduced that yields higher levels of sperm recovery and is rapid and reproducible (Möslein-Rossmiessl and Taubert 1989; Pickering et al. 1989; Pretorius and Kruger 1990). Because of these advantages, the SpermPrep technique could have significant effects in the manner that specimens are prepared and improved before their use in the various artificial (non-coital) reproduction procedures. The present study was designed to compare the traditional double sperm wash method to the new SpermPrep filtration technique when processing semen for IUI and their possible effects on pregnancy rates.

**Materials and Methods**

*Ejaculate collection*

Ejaculates were collected from all the males who participated in the current study. Ejaculates were collected with 2-4 days of abstinence each time. Patients collected their ejaculates either by using the seminal collection device (SCD) at intercourse (Quinlivan et al. 1982; Rogers et al. 1991) or via masturbation.

*Semen evaluation*

After semen samples were produced and completely liquefied (within 15-30 min), each
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specimen was evaluated according to standard procedures recommended by the World Health Organization (WHO) using a phase-contrast microscope (Russell and Rogers 1987). Semen measures included volume, sperm count per milliliter, percentage sperm motility, grade of sperm motility (Sofikitis et al. 1992), and sperm morphologic features. All seminal parameters were evaluated by the same technicians as per location. Spermatozoa were prepared either via the SpermPrep method or the double sperm wash method, evaluated and then used for IUI.

**SpermPrep filtration**

The SpermPrep was used according to the manufacturer’s specifications and instructions (ZBL, Inc.) and also according to previously described methodology (Zavos 1985, 1991, 1992a; World Health Organization 1987). It should be emphasized that proper standard laboratory techniques were employed in our laboratory during the filtration process and were applied similarly during the sperm wash. Those techniques included complete sterility and maintenance of all semen diluents, the SpermPrep filter, and all other materials within a temperature range of 30 to 35°C. Filtration was begun by placing the properly resuspended spermatozoa in the filter. At the end of filtration (10 to 15 min), the filtrate was centrifuged and resuspended in Ham’s F-10 or human tubal fluid (HTF), assessed and used for IUI purposes.

**Double sperm wash**

The semen was diluted 1:1 with the buffer of choice (HTF or Ham’s F-10), mixed and centrifuged at 300 × g for 10 min. This process was repeated and the generated pellet was gently resuspended in the buffer of (HTF or Ham’s F-10) choice, evaluated as previously described (Russell and Rogers 1987) and used for IUI purposes. Adequate numbers of motile spermatozoa were obtained from the double wash-resuspended aliquot for IUI to match the numbers of total motile sperm used in the SpermPrep reconstituted aliquot for IUI purposes.

**Patient group**

One hundred-forty eight couples participated in this study at two different locations in the USA (Table 1). The mean female age was 28.1 years (range 24 to 37), and the mean duration of the infertility was 3.8 years (range 1 to 9 years). Each couple underwent an examination that included a medical history, physical examination, semen analysis, evaluation of basal body temperature, serum progesterone determinations in the luteal phase of the menstrual cycle, postcoital tests, hysterosalphingogram, and laparoscopy. Couples with either tubal damages, subnormal semen samples according to the WHO (Russell and Rogers 1987), or immunological infertility were not included in the study.

Direct IUI insemination with controlled ovarian stimulation was mostly offered to couples with unexplained infertility, minimal endometriosis or with cervical factor before proceeding to any other form of artificial reproductive technology.

**Study design**

The couples were randomized before the first treatment cycle into two groups, one group being treated with sperm prepared by the traditional double wash method and the other with sperm prepared via the SpermPrep filtration method. In subsequent treatment cycles, the sperm preparation method was not alternated. The sperm parameters before and after preparation and the post-preparation parameters were compared. The pregnancy rates after direct IUI using either the double wash or SpermPrep preparations were also compared. In comparison of sperm parameters, the Student’s t-test was used. Biochemical pregnancies were only included in the calculations of sperm parameters.
Performance of IUI

Intrauterine insemination (IUI) was performed following proper ovulation predictions and the luteinizing hormone (LH) was determined in serum or urine (Ovusticks; Monoclonal Antibodies, Mountain View, CA, USA). In ovulation induction patients, at least two follicles of a minimum 15 mm were present. Ovulation was then induced by an injection of 10,000 IU human chorionic gonadotropin (hCG, Profasi; Serono, USA) and the inseminations were performed 22 to 42 hr (mean 38 hr) after the hCG injections. In cycles in which an endogenous LH surge was detected (Ovusticks), it was supported by an hCG injection (10,000 IU), and the insemination was performed the day after. Clinical pregnancies were always verified by ultrasound scanning in the 7th week of gestation. If the pregnancy could not be verified by ultrasound, it was regarded as biochemical.

Insemination

Patients undergoing IUI were instructed not to have intercourse for 2-3 days before the day of semen collection and insemination. A mean volume of 0.4 ml (range 0.3 to 0.5 ml) of washed sperm sample was aspirated into an 11.5 cm Tomcat catheter (Sherwood Medical, St. Louis, MO, USA) which had been fashioned to the natural curvature of the uterine cavity. The cervix was exposed with a bivalve speculum and the tip of the catheter was passed into the uterus until it lay about 0.5 cm from the top of the uterine cavity in the fundal region. The sperm fraction was then gently expelled and the catheter was gently withdrawn. The patients rested in a supine position for 20-30 min after the insemination was performed.

RESULTS

Clinical data of the patient population that participated in the current study is shown in Table 1. There were no differences in the ages between the two semen treatments of the husbands or wives, neither within each location nor between the two locations ($p > 0.05$). Similarly, no differences were noted in the time interval that the couples were trying to conceive between the two treatments, either within location or between locations ($p > 0.05$).

The semen samples were prepared via the SpermPrep method and by the double sperm wash technique. The different sperm parameters before and after preparation are presented in Table 2. Before preparation the semen parameters

<table>
<thead>
<tr>
<th>Location$^1$</th>
<th>Patients (n)</th>
<th>Husband’s age (years)</th>
<th>Wife’s age (years)</th>
<th>Years trying to conceive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^a$</td>
<td>28</td>
<td>32.7±2.6</td>
<td>27.6±3.1</td>
<td>4.1±0.8</td>
</tr>
<tr>
<td>1$^b$</td>
<td>29</td>
<td>31.3±3.4</td>
<td>29.2±2.8</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td>2$^a$</td>
<td>44</td>
<td>29.4±4.1</td>
<td>28.3±3.6</td>
<td>3.9±1.1</td>
</tr>
<tr>
<td>2$^b$</td>
<td>47</td>
<td>30.6±2.2</td>
<td>27.1±2.7</td>
<td>4.3±1.2</td>
</tr>
</tbody>
</table>

$^1$Location 1: University of Rochester Medical Center, NY, USA; Location 2: Andrology Institute of Lexington, Lexington, KY, USA.

$^a,b$Group 1: Semen prepared via SpermPrep method; Group 2: Semen prepared via double sperm wash method.
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for the 123 semen samples prepared by the SpermPrep and the 184 semen samples prepared via the double sperm wash differed insignificantly. After preparation with the SpermPrep method, the mean sperm density was lower ($p < 0.05$) than after preparation with the double wash technique. However, the percentage of motility and other qualitative measurements were higher with the SpermPrep method than with the double sperm wash (motility %: $76.3 \pm 6.8$ vs $52.1 \pm 7.1$, respectively; $p < 0.05$; % normal morphology: $81.6 \pm 5.0$ vs $51.4 \pm 6.8$, respectively; $p < 0.05$). To overcome the qualitative sperm differences noted between the two treatments, the IUI doses were standardized to reflect the similar motile sperm densities (MSD) used. However, greater total numbers of spermatozoa ($p < 0.05$) were inseminated in patients that received double washed sperm than

| Table 2. Sperm parameters before and after preparation with SpermPrep and double wash methods for IUI and numbers of total sperm and motile sperm inseminated between the two methods (means ± s.e.)¹ |
|-------------------------------|------------------|------------------|----------------------|
| Variable                      | Semen preparation method |                  |                      |
|                               | SpermPrep        | Double Wash      | Significance         |
| No. semen samples             | 123              | 184              |                      |
| MSD² (×10⁶/ml) before prep    | 112.3 ± 19.6     | 144.7 ± 20.7     | NS                   |
| MSD² (×10⁶/ml) after prep     | 60.8 ± 5.6       | 121.2 ± 15.6     | $p < 0.05$           |
| Total sperm inseminated      | 35.1 ± 6.2       | 56.4 ± 5.1       | $p < 0.05$           |
| Total motile sperm inseminated (×10⁶) | 26.7 ± 3.5     | 29.4 ± 3.7       | NS                   |

¹Values by location were pooled and shown only by treatment method.
²MSD, motile sperm density.

| Table 3. Distribution of pregnancies and cycle fecundity (cycles per pregnancy) within location and between location and total motile sperm (TMS) used at IUI after sperm preparation with the SpermPrep and double wash methods (means) |
|-----------------|-----------------|-----------------|-----------------|
| Location¹      | TMS² at IUI     | Patients (n = 148) | Cycles (n = 307) | Cycles per pregnancy |
| 1ᵃ              | 21.3            | 10/28 (35.7)ᶜ    | 10/ 49 (20.4)   | 4.9              |
| 1ᵇ              | 28.7            | 7/29 (24.1)      | 7/ 75 ( 9.3)   | 10.7             |
| 2ᵃ              | 32.3            | 16/44 (36.7)     | 16/ 74 (21.6)  | 4.6              |
| 2ᵇ              | 29.8            | 11/47 (23.4)     | 11/109 (10.1)  | 9.9              |

¹Location 1: University of Rochester Medical Center, NY, USA; Location 2: Andrology Institute of Lexington, Lexington, KY, USA.
²TMS: Total motile sperm used at IUI (×10⁶).
³ᶜGroup 1: Semen prepared via SpermPrep method; Group 2: Semen prepared via double sperm wash method.
ᶜValues in parentheses are percentages.
those inseminated with SpermPrep recovered sperm. This was necessary to overcome the lower motility ratio (51.4±6.8%) of the double washed sperm aliquots. The percentage recovery of spermatozoa was higher with the double wash (83.8%) than with the SpermPrep method (54.1%). The differences in the recorded recovery are shown in Table 2. The conception rates for sperm preparations with SpermPrep and double sperm wash by location and within location were different (p <0.05). The clinical pregnancy rates per cycle were statistically lower (p <0.05) in the double sperm wash treatment as compared to the SpermPrep treatment (20–22% vs 9–10%). Of significant clinical importance, almost twice as many cycles (10.3) were required in the double sperm wash treatment to achieve these pregnancies (9–10%) when compared to the SpermPrep group of patients that required only 4.8 cycles to achieve twice the level of pregnancies (20–22%; Table 3).

DISCUSSION

The main purpose of the study was to compare two different sperm preparation techniques, SpermPrep filtration and double sperm wash, in respect to sperm parameters after preparation and pregnancy rates (PR). During the preparation period, the SpermPrep method proved to be more consistent than the double sperm wash method and also yielded higher quality spermatozoa that could be used further for IUI purposes. In the present study in which the numbers of motile sperm inseminated (MSD) between the two treatments were similar, the SpermPrep prepared spermatozoa yielded a higher number of pregnancies. The surprising finding was that although similar numbers of motile spermatozoa were used during IUI in both treatment groups (by location), the SpermPrep prepared spermatozoa yielded higher pregnancies, which may be explained by the fact that a higher ratio of motile to dead sperm was used for the SpermPrep IUI’s than for the double sperm wash inseminations (76.1% vs 52.1%, respectively). Similar observations were noted with in-vitro fertilizing potential studies with SpermPrep and swim-up recovered human spermatozoa and their ability to penetrate zona-free hamster oocytes (Zavos 1992b) and the outcome of pregnancies using frozen-thawed semen and employment of IUI (Zavos and Centola 1990). The high recovery of motile sperm with the SpermPrep method achieved in this study, coupled with the beneficial effects on the outcome of pregnancies may indicate that the high quality and quantity of sperm recovered via the SpermPrep can render the SpermPrep method a viable technique in treating couples with male factor infertility as pointed out in other studies (Zavos and Goodpasture 1989; Zavos and Cohen 1990).

Direct IUI with SpermPrep prepared sperm seems to result in higher conception rates than IUI with double washed sperm. Most of the pregnancies occurred in the first treatment cycles. Even though the study did not contain randomized controls, this assertion in further supported by the fact that the PR per cycle was
higher after direct IUI with SpermPrep prepared sperm than in the observation cycles with double washed sperm. Thus, our data suggest that direct IUI with SpermPrep prepared sperm can increase the cycle fecundity and shorten the period of infertility, although whether the treatment increases the long-term PR per couple remains to be shown.

In conclusion, direct IUI with high quality sperm combined with controlled ovarian stimulation offers a simple and alternative method for the treatment of couples with unexplained infertility. The treatment of spermatozoa via the SpermPrep filtration method appears to increase the cycle fecundity and shorten the duration of infertility. The new sperm preparation method involving filtration of spermatozoa via the SpermPrep method resulted in a higher number of pregnancies when compared with the conventional double wash method, but the new method was also more convenient and yielded a higher ratio of motile spermatozoa. These improvements in the qualitative parameters of the spermatozoa obtained via the SpermPrep in this study, coupled with the ability of the SpermPrep to entrap and remove unstable DNA (single stranded DNA) spermatozoa (Zavos et al. 1991) which have been associated with lower fertilization rates (Zavos et al. 1992), could be the explanation for the higher fecundity noted in the patient group inseminated with these spermatozoa. Similar improvements in conception rates could be realized via the SpermPrep method with additional assisted reproductive technologies such as IVF, GIFT, ZIFT and others.

References


