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Can the method of sperm preparation for intrauterine insemination affect subsequent pregnancy rates? Comparison between the SpermPrep™ and the traditional double wash method

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ABSTRACT

Objective: To evaluate and compare the traditional double sperm wash method to the SpermPrep™ filtration technique when processing semen for intrauterine insemination (IUI) and their possible effects on pregnancy rates (PRs).

Design: A prospective study including ninety-one couples participating in this study. The couples underwent IUI under standardized conditions.

Setting: The Andrology Institute of Lexington and the Kentucky Center for Reproductive Medicine

Patients: Two hundred eighty-three couples undergoing infertility treatment were selected for this study. From those, ninety-one couples participated in this study.

Main Outcome Measures: Pregnancy rates per patient and pregnancies per cycle.

Results: The clinical PRs and clinical PRs per cycle were statistically superior when the SpermPrep™ semen preparation method was employed.

Conclusions: The results obtained suggest that IUIs with high quality sperm recovered via the SpermPrep™ filtration method combined with controlled ovarian hyperstimulation (COH) offer a simple alternative and superior mode of treatment for couples with unexplained infertility.

Key Words: Intrauterine insemination, semen preparation, SpermPrep™, pregnancy rates

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

A number of in-vitro manipulative techniques are currently available to remove the undesirable spermatozoa, debris and other factors and improve overall sperm quality in fresh semen specimens. The most popular ones include the simple sperm wash, swim-up or sperm rise methods (1), and

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swim-down or sedimentation type methods (2). Other popular methods include Ficoll centrifugation (3), Percoll density gradient (4,5), and Sephadex or glass wool fiber filtration (6,7). It should be emphasized, however, that with many of these sperm manipulative techniques the increase in sperm quality is often achieved at the expense of the total numbers of recovered spermatozoa that may not be necessarily advantageous for patients with various seminal deficiencies such as oligozoospermia. Also, equally important, the relatively long period of time required to perform these procedures is additionally detrimental since the life expectancy of low quality spermatozoa may be limited.

Recently, a new technique, the SpermPrep™ filtration method (ZDL, Inc., P.O. Box 23777, Lexington, KY 40523, USA.), has been introduced that yields higher levels of sperm recovery and is rapid and reproducible (6, 8-10). Because of these advantages, the SpermPrep™ technique could have a significant impact in the manner that specimens are prepared and improved before their use in the various assisted reproductive technologies (ART). 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. The specimens were collected with 2-4 days of abstinence each time. Patients collected their ejaculates via the use of the Male Factor Pak™, a semen collection device (SCD) at intercourse (11-13). The Male Factor Pak™ (MFP™; ZDL, Inc., Lexington, KY, USA) consists of a nonspermicidal condom made of polyurethane.

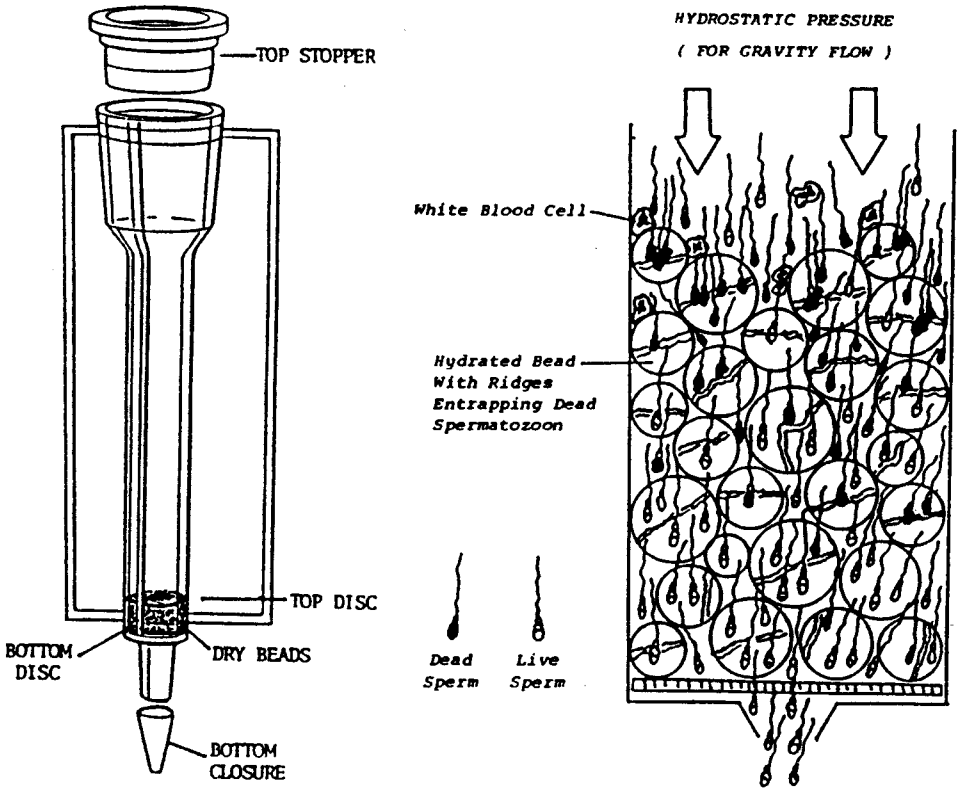

Figure 1. (Left) SpermPrep™ disposable semen filter column, (Right) diagrammatic illustration of the SpermPrep™ disposable semen filter column depicting the mode of action during sperm filtration.

Semen evaluation

After semen samples were produced and completely liquefied (within 15-30 min.), they were evaluated according to standard procedures recommended by the World Health Organization (WHO) using a phase contrast microscope (14). Semen parameters assessed included volume, sperm count per milliliter, percentage sperm motility, grade of sperm motility (15), and sperm morphologic features. All seminal specimens were evaluated by the same technician. 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 employed according to the manufacturer's specifications and instructions (ZDL, Inc.) and also according to previously described methodology (16-20). 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. These 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 begun by placing the properly resuspended spermatozoa in the filter (Figure 1). Following filtration (10 to 15 minutes), the filtrate was centrifuged, resuspended in SpermPrep™ medium (ZDL, Inc., Lexington, KY, USA), assessed and used for IUI purposes.

Double sperm wash

The semen was diluted 1:1 with the buffer of choice (SpermPrep™ medium), mixed and centrifuged at 300xg for 10 minutes. This process was repeated and the generated pellet was gently resuspended in the buffer of choice, evaluated as previously described (14) 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

Ninety-one couples participated in this study at the Andrology Institute of Lexington (Lexington, KY, USA) (Table 1). The mean female age was 27.7 years (range 24 to 35), and the mean duration of the infertility was 4.1 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 chart, serum progesterone determinations in the luteal phase of the menstrual cycle, postcoital tests, hysterosalpingogram, timed endometrial biopsy and laparoscopy. Couples with either tubal damage, subnormal semen samples according to the WHO (14), or immunological infertility, were not included in the study.

IUI with controlled ovarian stimulation (50 mg of Clomid only, daily) was offered to couples with unexplained infertility, minimal endometriosis, ovulatory dysfunction, or with cervical factor before proceeding to any other form of assisted reproductive technology (ART).

Table 1. Clinical data of patient populations studied (means±SE). [1]

Method employed	Number of Patients	e (years)		Years trying to conceive	
SpermPrep™	44	29.4	4.1	28.3 3.6	3.9 1.1
Double wash	47	30.6	2.2	27.1 2.7	4.3 1.2

[1]Andrology Institute of Lexington, Lexington, KY, USA.

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. The couples with various causes of infertility were pooled, since the causes of infertility between the two treatments was homogeneous. In subsequent treatment cycles, the sperm preparation method was not altered. We compared the sperm parameters. The Student's t-test was used for comparative purposes.

Performance of IUI

Intrauterine insemination (IUI) was performed following proper ovulation prediction using luteinizing hormone (LH) urine kits (Ovusticks; Monoclonal Antibodies, Mountain View, CA, USA). All COH patients were serially followed by ultrasound. The presence of at least one follicle with mean diameter of 15mm was the determining point to induce ovulation via IM Injection of 10,000 IU human chorionic gonadotropin (hCG, Profasi; Serono, USA). The inseminations were performed 22 to 42 hours (mean 38 hours) after the hCG injection. In cycles in which an endogenous LH surge was detected (Ovusticks), the insemination was performed the following day. Clinical pregnancies were ultrasonographically verified by the presence of fetal heart activity during 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 to abstain from 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 is fashioned to the natural curvature of the uterine cavity. The cervix was exposed with a bivalve speculum and the tip of the catheter was introduced into the uterus until it lay approximately 0.5 cm from the fundus of the uterine cavity. The sperm fraction was slowly expelled and the catheter was gently withdrawn. The patients rested in the supine position for 20-30 minutes after the insemination was performed.

RESULTS

Clinical data of the patient population that participated in the current study is shown in Table 1. There was no difference in the age of the husbands or wives ($P>0.05$) between the two semen treatment groups. Similarly, no difference was noted in the time interval that the couples were trying to conceive between the two treatment groups ($P>0.05$).

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.[1]

Variables assessed	Semen Preparation Methods		Significance
	SpermPrep™	Double Wash	
Number of semen samples	74	109	-
MSD[2] ($x10^6$/mL) before prep	109 17.2	145.6 21.6	NS
MSD[2] ($x10^6$/mL) after prep	61.2 4.7	125.1 16.6	P<0.05
Total sperm inseminated ($x10^6$)	34.2 5.3	57.3 4.9	P<0.05
Total motile sperm inseminated ($x10^6$)	26.6 3.6	29.8 4.1	NS

[1]Values are means (±SE) and compared between the two semen preparation methods.
[2]MSD: motile sperm density.

Table 3. Distribution of pregnancies, cycle fecundity (cycles per pregnancy) and total motile sperm (TMS) used at IUI after sperm preparation with SpermPrep™ and double wash methods (means).[1]

Method used	TMS[2] at IUI	Pregnancy rates		
		Patients (n=91)	Cycles (n=183)	Cycles per pregnancy
SpermPrep™	26.6 3.6	26/44 (36.7)[a]	16/84 (21.6)[b]	4.6[b]
Double wash	29.8 4.1	11/47 (23.4)	11/109 (10.1)	9.9

[1]Andrology Institute of Lexington, KY, USA.
[2]TMS: Total motile sperm used at IUI ($x10^6$).
[a]Values in parentheses are percentages.
[b]Significant differences between methods used ($p<0.05$).

The semen samples were prepared via the SpermPrep™ method or the double sperm wash technique. The sperm parameters before and after preparation are presented in Table 2. There were no statistically significant differences ($p<0.05$) in semen parameters between the 74 semen samples prepared by the SpermPrep™ and the 109 semen samples prepared via the double sperm wash. 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 normal morphology measurements (Table 2) were higher with the SpermPrep™ method than with the double sperm wash (motility %; 77.8 ± 6.3 vs. 57.1 ± 6.7, respectively; $P<0.05$; % normal morphology: 82.3 ± 5.1 vs. 49.7 ± 7.0, 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 those inseminated with SpermPrep™ recovered sperm. This was necessary to overcome the lower motility ratio (49.7 ± 6.3%) of the double washed sperm aliquots. The percentage recovery of spermatozoa was higher ($p<0.05$) with the double wash (85.9%) than those following IUI with sperm prepared via the SpermPrep™ method ($P<0.05$). The clinical pregnancy rates per cycle were statistically lower ($P<0.05$) in the double sperm wash treatment as compared to the SpermPrep™ treatment (21.6% vs. 10.1%). Of significant clinical importance, almost twice as many cycles (10.1cycles) were required in the double sperm wash treatment to achieve these pregnancies (9.9%) when compared to the SpermPrep™ group of patients that required only 4.6 cycles to achieve twice the level of pregnancies (21.6%; 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) following IUI. 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 for IUI. In the present study in which the numbers of motile sperm inseminated 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, the SpermPrep™ prepared spermatozoa resulted in higher pregnancy rates, which may be explained by the fact that a higher ratio of motile to dead sperm was used for the SpermPrep™ double sperm wash inseminations (77.8% vs. 49.7%, respectively). Similar observations were noted in studies using human spermatozoa recovered via the SpermPrep™ filtration method or swim-up in regards to their in-vitro fertilizing potential, their ability to penetrate zona-free hamster oocytes (20) and pregnancy rates

following IUI with frozen-thawed spermatozoa (12). Similarly, spermatozoa recovered via the SpermPrep™ filtration and Percoll methods were proven to be of similar qualitative and quantitative characteristics (21). The high recovery of motile sperm with the SpermPrep™ method achieved in this study, coupled with the beneficial effect on pregnancy rates may indicate that the high quality of sperm recovered via the SpermPrep™ can render this method a viable and convenient technique in treating couples with male factor infertility as pointed out also in other studies (22, 23).

Inseminations (IUI) with SpermPrep™ prepared spermatozoa seem to result in higher conception rates than IUI with double washed sperm. Most of the pregnancies occurred in the first treatment cycle. Even though the study did not contain randomized controls, this assertion is further supported by the fact that the PR per cycle was higher after IUI with SpermPrep™ prepared sperm than in cycles with double washed sperm. Thus, our data suggests that IUI with SpermPrep™ prepared sperm can increase the cycle fecundity and shorten the time period of infertility treatment. It should be noted that all inseminations in this study were performed by the same physician (Inseminator) in order to avoid any possible variations due to the human factor and IUI response (23).

In conclusion, 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 treatment. The new sperm preparation method involving filtration of spermatozoa via the SpermPrep™ resulted in a higher number of pregnancies when compared with the conventional double wash method. Furthermore, this new method was more convenient and yielded a higher ratio of motile spermatozoa. Also, those improvements achieved via the SpermPrep™ method were similar to improvements noted when Percoll was used and compared in a previous study (22). The results in this study indicate that these improvements in the qualitative parameters of the spermatozoa obtained via the SpermPrep™ method, coupled with the ability of the SpermPrep™ to entrap and remove unstable DNA (single stranded DNA) spermatozoa (9) which have been associated with lower fertility rates, could be the explanation for the higher fecundity noted in the patient group inseminated with these spermatozoa. Similar improvements in conception rates could also be realized via the SpermPrep™ method in conjunction with other assisted reproductive technologies (ART). Further studies are currently underway to investigate the employment of the SpermPrep™ filtration method

REFERENCES

1. Russell DL, Rogers BJ. Improvement in the quality and fertilization potential of human sperm population using the rise technique. J Androl 1989;8:25-33.
2. Dmowski WP, Gaynor L, Rao R, Lawrence M, Scommegna A. Use of albumin gradients for X and Y sperm separation and clinical experience with male sex preselection. Fertil Steril 1979;31:52-7
3. Kaneko S, Moriwaki C, Sato H. Development of multiple exposure photography method for analysis of sperm motility and preparation of washed sperm with Ficoll density gradient. Jpn J Fertil Steril 1980;25:491-3.
4. McClure RD, Nunes L, Tom R. Semen manipulations improved sperm recovery and function with a two-layer Percoll gradients. Fertil Steril 1989;51:874-7.
5. Pickering SJ, Fleming TP, Braude PR, Bolton VN, Gresham GAG. Are human spermatozoa separated on a Percoll density gradient safe for therapeutic use? Fertil Steril 1989;51:1024-29.
6. Centola GM, Zavos PM. Qualitative/quantitative improvements in post-thaw human semen using SpermPrep™. J Assisted Reproductive Technology-Andrology 1991;2:335-9.
7. Katayama KP, Stehilk E, Jayendran RS. In vitro fertilization outcome: glass wool-filtered sperm versus swim-up sperm. Fertil Steril 1989;52:670-2.
8. Free D, Stutts L, Merrymen D, Stringfellow E, Houserman V, Honea K. A comparison of donor semen processing techniques for use in intrauterine inseminations and their corresponding pregnancy rates. Proceedings of the 47th Annual Meeting of the American Fertility Society; October 21-24; Orlando, (FL). AFS Program Supplement, 1991:S91.
9. Sofikitis N, Miyagawa I, Zavos PM. The SpermPrep™ filtration method selectively en traps single strand DNA spermatozoa. Jpn J Fertil Steril 1992;37:10-13.
10. Zavos PM. Preparation of human frozen-thawed specimens using the SpermPrep™ filtration method: Improvements over the conventional swim-up method. Fertil Steril 1992;57:1326-30.

11. Zavos PM. Characteristics of human ejaculates collected via masturbation and a new Silastic seminal fluid collection device (SCD). Fertil Steril 1985;43:491-2.
12. Zavos PM. Selection de spermatozoides viables a partit d' echantillons de spermes humains ongeles-decongeles: comparison de la methode du "swim-up" et d'une nouvelle methode de filtration: le SpermPrep™. Contracept Fertil Sex 1991;19:293-7.
13. Zavos PM, Goodpasture JC. Clinical improvements of specific seminal deficiencies via intercourse with a seminal collection device versus masturbation. Fertil Steril 1985;51:190-3.
14. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 2nd edition. Cambridge, The Press Syndicate of the University of Cambridge, 1987:62.
15. Zavos PM, Cohen MR. The pH of cervical mucus and the postcoital test. Fertil Steril 1980;34:234-6.
16. Zavos PM. Amelioration des caracteristiques qualitatives des spermatozoides humains cryoconserves apres recuperation par la methode de filtration SpermPrep™ il. Contracept Fertil Sex 1992;20:541-5.
17. Zavos PM, Centola GM. Qualitative and quantitative improvements in human spermatozoa recovered via the swim-up and a new semen filtration method. Infertility, 1990;13:25-34.
18. Zavos PM, Sofikitis N, Toda T, Miyagawa I. Improvements in qualitative characteristics of cryopreserved human spermatozoa following recovery via the SpermPrep™ II filtration method. Tohoku J Exp Med 1991;163:283-90.
19. Zavos PM, Sofikitis N, Toda T, Miyagawa I. Selection and preparation of human spermatozoa for artificial insemination using the new and improved SpermPrep™ II filtration method. Jpn J Fertil Steril 1992;37:14-9.
20. Rogers BJ, Wamil B, Zavos PM. Comparison of the fertilizing potential of human spermatozoa processed by Swim-up or Sephadex filtration columns. Proceedings of the Seventh World Congress on IVF and Assisted Procreation 1991:318.
21. Horvath PM, Boher M, Shelden RM, Kemmann E. The relationship of sperm parameters to cycle fecundity in superovulated women undergoing intrauterine insemination. Fertil Steril 1989:52:288-294.
22. Check JH, Zavos PM, Katsoff D, Kiefer D. Effects of Percoll discontinuous density gradients vs. SpermPrep™ II vs. Sephadex gel filtration on semen parameters. Tokoku J Exp Med 1993; 12:506-512.
23. Moslein-Rossmeissi S, Taubert HD. Male subfertility and the outcome of intrauterine insemination. Andrologia 1989;21:519-23.
24. Zavos PM, Zarmakoupis-Zavos PN, Correa JR, Aboubdalla M, Aslanis P. Variations in pregnancy rates following intrauterine insemination among infertility centers: can the inseminators make a difference? Middle East Fertil Soc J 1997; 2:24-29.

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