FERTILITY AND STERILITY®
VOL. 74, NO. 4, OCTOBER 2000

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Use of the Multi-ZSC one-step standardized swim-up method: recovery of high-quality spermatozoa for intrauterine insemination or other forms of assisted reproductive technologies

The human ejaculate contains a mixture of motile, nonmotile, and agglutinated spermatozoa and various types of debris. Today, a number of in vitro manipulative techniques are currently available to remove the undesirable spermatozoa, debris, and other factors and improve the overall sperm quality in fresh semen specimens. However, all of the techniques that are available today allow the recovery of only one sperm fraction of similar qualitative characteristics to be recovered and used further in assisted reproductive technologies (ARTs).

Recently, a new technique, the Multi-ZSC (ZDL, Inc., P.O. Box 23777, Lexington, KY 40523), that is rapid, reproducible, and enables the recovery of various subpopulations of high-quality spermatozoa was introduced. Because of these advantages, the Multi-ZSC technique could have a significant impact in the manner that specimens are prepared and improved before their use in the various ARTs and IUI procedures. The current study was designed to evaluate the efficacy of the Multi-ZSC method for sperm preparation from normospermic patients and to assess the possible enhancement of the quality of the subpopulations of spermatozoa prepared and recovered via the Multi-ZSC.

Ejaculates were collected from 20 men who were referred to the Andrology Institute of America laboratory facilities for male infertility examination. Ejaculates were collected with use of the Male Factor Pack (MFP; ZDL, Inc.) at intercourse after 4 days of abstinence (1). The experimental protocol of the current study was reviewed and approved by the Institutional Review Board at the Andrology Institute of America. After semen liquefaction, each specimen was evaluated for volume, sperm count per milliliter, percentage of sperm motility, grade of sperm motility (scale of 0–4), sperm morphologic features, and the percentage of spermatozoa reactive to the hypoosmotic swelling (HOS) test, according to the World Health Organization (WHO). All seminal parameters were evaluated by the same technician who was unaware of the conditions.

The Multi-ZSC procedure was performed according to the manufacturer's specifications by placing a 2.0-mL volume of semen into the conical cylinder of the Multi-ZSC. A 1.0-mL volume of SpermPrep media (ZDL, Inc.) was placed into each compartment of the Multi-ZSC column, completely saturating the areas around each of the four cones (periconical) and a certain level of the area over the top cone of the Multi- ZSC column (2). The prepared Multi-ZSC was capped and incubated (35°C) for 1 hour to allow high-quality, motile spermatozoa to swim up from the semen side (conical cylinder) up and into the top layers of each coned compartment filled with the media. At the end of the incubation period, the media that was overlayered around and on top of the semen and in each of the conical cylinders was removed by compartment and the recovered spermatozoa from each compartment were placed in separate test tubes and assessed as previously described.

Received December 30, 1999; revised and accepted April 17, 2000.
Presented in part at the 12th Annual Meeting of the Middle East Fertility Society, Amman, Jordan, November 18–20, 1998.
Reprint requests: Panayiotis M. Zavos, Ed.S., Ph.D., 607 W. P. Garrigus Bldg., University of Kentucky, Lexington, Kentucky 40546 (FAX: 606-278-6906).

0015-0282/00/$20.00
PII S0015-0282(00)01488-6

The results obtained in this study are summarized in Table 1. The semen specimens used in this study were considered normospermic according to WHO standards. The total sperm recovery from specimens processed via the Multi- ZSC method from all four compartments represented 41.4% of the initial number of spermatozoa in the unprocessed specimens. Sperm specimens recovered via the Multi- ZSC method were qualitatively superior ($P<.05$) compared with the unprocessed, raw specimens, independent of the compartment recovered. Furthermore, statistically significant differences were noted in the numbers of sperm recovered and total functional sperm fraction (TFSF) values ($P<.05$) among the four compartments of the Multi-ZSC (Table 1).

The TFSF values for the four (bottom to top) Multi-ZSC specimens recovered were 16.6, 6.8, 5.4, and 3.4×10^6 spermatozoa per mL, respectively (Table 1). Of greater significance, the overall quality of spermatozoa improved the most ($P<.05$) when recovered from the top compartments of the Multi-ZSC compared with those recovered from the bottom compartments ($P<.05$). The morphologic profile along with the membrane integrity measurements (HOS-test) of the spermatozoa recovered from the top compartment (fraction 4) were the highest and quite superior ($P<.05$) compared with those recovered from the other three compartments.

TABLE 1

Sperm quantitative and qualitative characteristics of specimens assessed before and after processing via the Multi-ZSC method.

	Semen treatments (fractions recovered)				
Semen parameters (n = 20)	Control	Fraction 1[a]	Fraction 2	Fraction 3	Fraction 4
Volume recovered (mL)	3.1 ± 0.4[h]	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
Concentration ($\times 10^6$/mL)	103.6 ± 9.7	21.8 ± 4.6[d]	8.3 ± 2.2[e]	6.1 ± 1.2[e]	4.0 ± 0.3[g]
Motility (%)	61.3 ± 4.5	93.7 ± 4.1[d]	98.9 ± 1.0[d]	100.0 ± 0.0[e]	100.00 ± 0.0[e]
Grade (0–4)	3.3 ± 0.3	3.7 ± 0.2[d]	3.8 ± 0.1[d]	4.0 ± 0.0[e]	4.0 ± 0.0[e]
Morphology (% normal)	58.5 ± 5.6	75.1 ± 6.8[d]	83.1 ± 4.1[d]	88.3 ± 3.2[e]	92.2 ± 2.0[f]
HOS[b] (%)	51.2 ± 9.6	69.7 ± 4.2	80.2 ± 1.3	86.0 ± 0.9	91.0 ± 0.5
TFSF[c] ($\times 10^6$/mL)	115.2 ± 6.1	16.6 ± 1.2	6.8 ± 0.3	5.4 ± 0.2	3.4 ± 0.1[d]

[a] Lowest level closest to the bottom cone containing the semen in the Multi-ZSC.

[b] HOS = hypoosmotic swelling test (% swollen spermatozoa).

[c] TFSF = total functional sperm fraction (total sperm concentration ($\times 10^6$) × % motility × % normal morphology).

[d–g] Statistically significant differences noted among the Multi-ZSC fraction values ($P<.05$).

[h] Statistically significant differences noted between all control and experimental sperm characteristics assessed ($P<.05$).

Zavos. Sperm improvements via the Multi-ZSC. Fertil Steril 2000.

All semen manipulative techniques aim to select spermatozoa that have a higher fertilizing capacity than the overall spermatozoa present in the original ejaculate. With the advent of IVF and other ARTs, it is considered important to use the best spermatozoa available from a semen sample. The retrieved spermatozoa are normally selected on the basis of motility, progressive motility, and morphological characteristics (3). Spermatozoa retrieved on the basis of their qualitative characteristics yield higher fertilization rates and pregnancy rates (4).

The data presented in this study indicate that the Multi-ZSC sperm selection method yielded significant improvements in the assessed qualitative characteristics compared with the control, unprocessed specimens ($P<.05$). In addition, the Multi-ZSC method, because of its unique design, yielded subpopulations of spermatozoa of different qualitative characteristics. When the TFSF value was calculated for all four compartments, an inclusive term that takes into consideration the total sperm count, percent motility and percent morphologically normal spermatozoa, then the sperm values were quite satisfactory for use in any ART procedure that could yield adequate fertilization rates.

Because of its unique design, the newly introduced Multi-ZSC could enable the user to layer the semen and the media in the various compartments with ease and a low margin of error. The same seems to be true during the recovery of the overlayered media from the compartments that contain the motile, healthy population of spermatozoa. The Multi-ZSC technique also enabled the harvesting of 100% of the overlayered medium, and most importantly, the medium closest to the underlayered semen, which maximized the number of sperm recovered. This technique could be extremely beneficial for patients with spermatogenic deficiencies and also could be time-saving for normospermic patients.

In addition, the Multi-ZSC method is a standardized technique that would enable the clinician and the researcher, by considering the numbers and quality of spermatozoa in the various compartments, to draw various inferences and conclusions during comparisons between different specimens from the same patient or from different patients with various spermatogenic parameters. In addition, the generated results suggest that the Multi-ZSC technique could be beneficial for selecting and maximizing the recovery of sperm of high qualitative characteristics that could be used further in the various forms of ARTs, especially ICSI when indicated.

Panayiotis M. Zavos, Ed.S., Ph.D.[a,b,c]
Michel Abou-Abdallah, M.D.[d]
Pavlos Aslanis, M.D.[a]
Juan R. Correa, Ph.D.[b,e]
Panayota N. Zarmakoupis-Zavos, M.D.[a,b,c]
Greek-American Andrology Institute of Athens, Athens, Greece[a]; Andrology Institute of America, Lexington, Kentucky[b]; Kentucky Center for Reproductive Medicine and In Vitro Fertilization, Lexington, Kentucky[c]; Clinique Dr. Rizk, Beirut, Lebanon[d]; and Centro de Fertilidad del Caribe, Rio Padres, Puerto Rico[e]

References

1. Zavos PM, Kofinas GD, Sofikitis NV, Zarmakoupis PN, Miyagawa L. Differences in seminal parameters in specimens collected via intercourse and incomplete intercourse (coitus interruptus). Fertil Steril 1994;61:1174–6.
2. Correa JR, Zarmakoupis-Zavos PN, Zavos PM. Quantitative and qualitative characteristics of frozen-thawed bovine spermatozoa recovered via a conventional and a standardized swim-up technique. Tohoku J Exp Med 1997;180:257–64.
3. Zavos PM. Preparation of human frozen-thawed seminal specimens using the SpermPrep filtration method: improvements over the conventional swim-up method. Fertil Steril 1992;57:1326–30.
4. Zarmakoupis-Zavos PN, Zavos PM. Can the method of semen preparation for intrauterine insemination affect subsequent pregnancy rates? Comparison between the SpermPrep and the traditional double wash method. Middle East Fertil Soc J 1998;3:164–70.