

Filtration of spermatozoa through L4 membrane: a new method

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Objective: To explore the possibility of using the L4 membrane filtration device for processing of spermatozoa for intrauterine insemination and other assisted reproductive techniques.

Design, Setting, Patients: Semen samples from 15 patients attending an infertility clinic of a tertiary referral institution and from seven fertile donors were used for the study.

Main Outcome Measures: The effect of L4 membrane filtration was studied on the following semen parameters: sperm motility, velocity, percentage of normal and abnormal sperm forms, and percentage and concentration of round cells in semen.

Results: The statistical analysis of data from both the subfertile patient population and the normal fertile donor group showed similar improvement in semen quality after L4 membrane filtration. Therefore, the results for these two groups were combined for statistical purposes. Our findings demonstrate a significant increase in normal sperm forms and a decrease in abnormal forms after filtration of specimens through the L4 membrane. In all subjects, the mean sperm motility increased from 47% to 77% ($P < 0.0001$); velocity increased from 30 to 48 $\mu\text{m}/\text{seconds}$ ($P < 0.0001$); normal sperm forms increased from 53% to 84% ($P < 0.0001$); abnormal forms decreased from 28% to 10% (head defects) ($P < 0.0001$); neck defects from 3.5% to 1% ($P < 0.0006$); tail defects from 8% to 3.5% ($P < 0.005$); and undifferentiated round cells decreased from 6% to $<1\%$ ($P < 0.002$) after filtration. The concentration of round cells counted by the Makler chamber (Sefi Medical, Haifa, Israel) decreased significantly from $6 \times 10^6 /\text{mL}$ to $0.2 \times 10^6 /\text{mL}$ ($P < 0.0003$). Similarly, white blood cell counts measured by the Endtz test also decreased from $3.4 \times 10^6 /\text{mL}$ to $<0.1 \times 10^6$ ($P < 0.043$).

Conclusion: We conclude that the filtration of the semen specimens through L4 membrane results in a significant improvement in semen parameters and should be considered as an adjunct in sperm processing. Fertil Steril 1991;56:1162-5

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This study was undertaken to explore the feasibility of using the L4 membrane filtration device (Pall Biosupport Corporation, New York, NY) to process spermatozoa for intrauterine insemination (IUI) or other assisted reproductive techniques. The L4 membrane is a fibrous polyester sheet whose surface characteristics have been modified to retain, by adsorption to the fiber surfaces, $\geq 99.9\%$ or more of white blood cells (WBCs) from blood passing through it. The red blood cell and platelet content of the blood passes through freely, whereas the leukocytes are attached throughout the upper portions of the medium. The L4 membrane is approved by the United States Food and Drug Administration for blood transfusion and has been used previously only to separate WBCs from human serum. We report the first use, to our knowledge, of the L4 membrane to filter semen specimens from suspected subfertile men with male factor infertility and from proven fertile donors. In the present experiment, we adapted the L4 leukocyte adsorption membrane to filter morphologically normal sperm forms from ejaculates containing a large number of abnormal spermatozoa, undifferentiated round cells, and WBCs. A highly significant increase in the percentage motility, velocity, and percentage of normal sperm forms and a reduction in morphologically abnormal sperm forms, undifferentiated round cells, and WBCs were observed after filtration through the L4 membrane.

MATERIALS AND METHODS

Semen Collection and Analyses

Semen samples were obtained by masturbation from seven proven fertile donors and 15 suspected subfertile patients, after 48 hours of sexual abstinence, into sterile wide mouthed plastic containers. The specimens were allowed to liquefy at 37°C for 30 minutes. Semen analyses were performed, using an automated, computerized system, Cell Soft 2000 (Cryo-Resources, New York, NY). A drop of semen (5 μL) was placed on a Makler chamber (Sefi Medical, Haifa, Israel), and sperm concentration and motility were measured.

The following parameter settings were used: frames analyzed:15; frames/sec: 30; video standard (A/E): A; minimum sampling motile:1; velocity:7; maximum velocity ($\mu\text{m}/\text{sec}$):200; threshold velocity: 10; threshold gray level:130; cell color:white; pixel scale ($\mu\text{m}/\text{sec}$):0.688; dilution factor:1.0; cell size range (pixels):low 5, high 25.

Seminal smears were prepared from liquefied specimens for assessment of sperm morphology by standard methods and stained by Hemacolor (EM Diagnostics, Inc., Gibbstown, NJ). One hundred cells were scored according to World Health Organization guidelines¹ for normal forms; head, neck and tail defects; and undifferentiated round cells at 400X magnification (Olympus BH2, Tokyo, Japan).

L4 Membrane Filtration Procedure

The L4 membrane was cut into 16-mm-diameter disks to fit in a 12.5-mL Eppendorf pipette tip. Two disks were placed at the bottom of the pipette tip. Semen specimens were mixed with 5.0 mL of human tubal fluid medium (Irvine Scientific, Santa Ana, CA), supplemented with 10% heat-inactivated fetal bovine serum (vol/vol). The medium was subsequently filter sterilized (Millipore Corporation, Bedford, MA), and the semen-human tubal fluid suspension was gently vortexed and passed through mounted disks by gravity. The specimens were rinsed with 4.0 mL of fresh medium and passed through the L4 membrane to collect the remaining motile spermatozoa from its surface. Specimens were filtered, then centrifuged at 600 X 9 for 5 minutes (International Equipment Company, Needham, MA). Supernatants were carefully aspirated by a Pasteur pipette and discarded. Sperm pellets were resuspended in 0.5 mL of medium, and complete semen analyses were again performed. Slides for morphological study were prepared from the final resuspended specimens.

Round Cell Determination

The concentration of undifferentiated round cells was determined by observation of a drop of specimen with use of a Makler chamber, under a

phase-contrast microscope (200X). This procedure was performed on the original ejaculate and on each specimen processed through the L4 membrane filtration system. The presence of granulocytes was identified via the Endtz test²: 20 μ L of liquefied specimen was placed in a Corning 2.0-mL cryogenic vial; 20 μ L of phosphate-buffered saline and 40 μ L of benzidine solution were added. The mixture was vortexed and allowed to sit at room temperature for 5 minutes. Peroxidase-positive WBCs staining dark brown were counted in a Makler chamber under the 20X bright field objective. Cells staining dark brown were counted in all 100 squares of the grid. The results after correction were recorded as 1×10^6 /mL.

Statistical Analysis

Statistical analysis included comparison by Student's paired t-test of the percentage of motile sperm, velocity, sperm morphology scores, and undifferentiated round cell concentration before and after processing by the L4 membrane technique. Because the concentration of seminal granulocytes is not normally distributed in either donor or suspected subfertile patient populations,³ nonparametric Wilcoxon's signed rank test was used to detect differences before and after L4 membrane filtration. All statistical evaluations were done using the Stat-view II program (Abacus Concepts, Inc., Berkeley, CA) on a Macintosh SE-30 personal computer.

RESULTS

Both suspected sub fertile patients and fertile donors exhibited similar increases in semen quality after L4 membrane filtration. Thus, the data from these two groups were combined for statistical purposes.

Sperm Motility, Velocity, Recovery, and Morphology

A significant increase in motility and velocity of spermatozoa was observed after filtration of semen

specimens through the L4 membrane. Sperm motility increased from a mean of 47% to 77% ($P < 0.0001$) and velocity from 30 to 48 μ m/sec ($P < 0.0001$) after filtration (Fig. 1). The mean recovery rates of total and motile sperm count after filtration were 30% and 43%, respectively. The filtration of sperm specimens from proven fertile donors and suspected subfertile men ($n = 22$) resulted in a significant increase in percentage of normal sperm forms from a mean of 53% to 84% ($P < 0.0001$). The presence of abnormal sperm forms showed a significant decrease after filtration by the L4 membrane: head defects decreased from 28% to 10% ($P < 0.0001$); neck defects from 3.5% to 1% ($P < 0.0006$); tail defects from 8% to 3.5% ($P < 0.005$); and undifferentiated round cells from 6% to $< 1\%$ ($P < 0.002$) (Fig. 2).

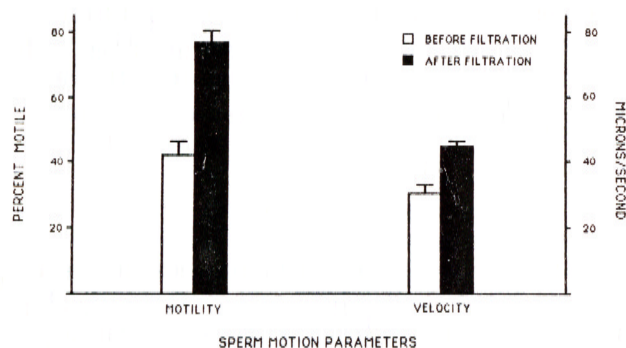


Figure 1 Percentage motility and velocity values of patient and donor specimens before and after filtration by L4 membrane (mean \pm SE).

Round Cells and WBCs

The concentration of undifferentiated round cells (immature germ cells and WBCs) counted by Makler chamber showed a significant decrease from a mean of 6×10^6 /mL to 0.2×10^6 /mL after filtration by the L4 membrane ($P < 0.0003$) (Fig. 2). The concentration of peroxidase-positive granulocytes also showed a significant decrease from 3.4×10^6 /mL to $< 0.1 \times 10^6$ /mL ($P < 0.043$) (Fig. 2).

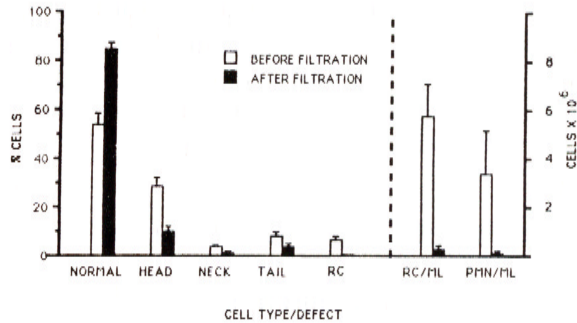


Figure 2 Percentage morphologically normal and abnormal (head, neck, tail defect, undifferentiated round cells [RC]) cell types in semen and concentration of undifferentiated round cells and white blood cells (polymorphonuclear leukocytes [PMN]) of patient and donor specimens before and after filtration by L4 membrane (mean \pm SE).

DISCUSSION

In cases of male infertility, cervical factor infertility, or unexplained infertility in which IUI appears more advantageous than intracervical or vaginal insemination, in vitro preparation of the semen specimen is required to avoid cramping and infection.⁴ In IUI we attempt to transfer into the uterus a high concentration of motile, seminal plasma-free, and viable sperm in a small volume of culture medium. Different methods are available for washing spermatozoa free of seminal plasma, such as sperm washing and swim-up,⁵ layering and swim-up,⁶ albumin gradient separation,⁷ glass wool separation,⁸ glass bead column separation,⁹ and Percoll gradient separation.¹⁰ However, each of the above techniques has some limitations. Swim-up, layering, albumin gradient, and glass bead column methods result in recovery of a low total count of motile spermatozoa.¹¹ In glass wool columns, all the seminal plasma is not removed, and the retention of glass fiber material in the sample and ultrastructural damage to the membrane and acrosome of the sperm can be a problem.¹²

Percoll density gradient separation can cause damage to sperm membranes¹³ and a decrease in percentage of morphologically normal sperm.¹¹ The ideal sperm preparation technique for IUI is that with the capacity to concentrate from the original ejaculate the largest number of morphologically normal, viable sperm of high motility in the smallest volume of physiological culture medium, free of seminal plasma, leukocytes, and bacteria.

The purpose of sperm-washing procedures is to obtain sperm populations that have motility superior to that of spermatozoa in native semen and thus to increase the chances of fertilization and conception. The sperm-washing procedures, however, do not imply any type of sperm selection. Repeated washings and centrifugations reduce sperm motility and increase necrospermia.¹⁴ Our results demonstrate that sperm filtration by the L4 membrane achieves a good yield of morphologically normal spermatozoa with enhanced motility and velocity. In addition, the number of WBCs in semen is significantly reduced by the filtration method. Excessive leukocytospermia is associated with poor semen parameters and sperm function.¹⁵ This procedure takes only 15 to 20 minutes, substantially less time than any other sperm-washing method. Unlike other methods, the current technique uses only a single centrifugation step to concentrate sperm and should reduce any kind of ultrastructural damage to the sperm. To assess the effect of filtration on the sperm's fertilizing ability, we are currently evaluating the functional status of filtered sperm by a battery of assays: hypo-osmotic swelling test, hamster egg penetration test, bovine cervical mucus penetration test, acrosome reaction assay, and acrosin measurement. In summary, sperm filtration by L4 membrane is a simple, fast, and a convenient method of preparing sperm for IUI and may also be suitable for other purposes, such as in vitro fertilization or gamete intrafallopian transfer.

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