

Improved Motile Sperm Recovery by a Hyperosmotic Percoll Gradient

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Submitted: February 18, 1997

Accepted: March 24, 1997

Purpose: Our purpose was to investigate whether a new, relatively hyperosmotic Percoll gradient, Enhance-S, can improve total motile sperm recovery rates compared with the commonly used Percoll gradient Perception.

Methods: Semen specimens from each of 17 donors were divided into two equal aliquots. One part was washed using Percoll Perception, while the other was prepared using Percoll Enhance-S.

Results: Compared to the unwashed specimen, sperm motion characteristics {motility and velocity) improved significantly after Percoll separation using either the Perception or the Enhance-S gradient. There was no difference in motility or velocity in spermatozoa recovered after wash with either of the two preparations. However, the total motile sperm recovery was significantly higher using the Percoll Enhance-S gradient than with the Percoll Perception gradient ($P < 0.0024$).

Conclusion: The new Percoll Enhance-S gradient provides significantly more total motile sperm than the Percoll Perception gradient.

KEY WORDS: assisted reproductive techniques; hyperosmotic; Percoll; semen; sperm.

INTRODUCTION

The ideal semen processing technique should result in the selective recovery of good-quality motile sperm while maintaining a high total yield of recovered sperm. Several procedures for retrieving motile sperm from the ejaculate have been described, including glass-wool filtration (1), albumin gradients (2), Percoll gradients (3,4), and swim-up procedures (5). It has been demonstrated that use of the discontinuous Percoll gradient technique produces a relatively large fraction of motile sperm with an improved fertilizing capacity and increased longevity (6). Specimens prepared by Percoll separation before freezing were found to select spermatozoa that retain motility for up to 24 hours (7).

Percoll preparations are not currently recommended for human use in the United States due to safety concerns with the therapeutic use of Percoll in the clinical setting, and the Enhance-S formulation is subject to the same restrictions. However, it should be noted that these concerns regarding the endotoxin content of Percoll during assisted reproductive procedures are not supported by results with mouse embryo bioassays (8) and that Percoll is still widely applied in the research setting. Moreover, knowledge gained by the use of hyperosmotic sperm preparations in an effort to improve the recovery of motile sperm may be applied to the development of newer sperm separation techniques.

A number of variations of the discontinuous Percoll gradient technique have been described. Because the semen processed by Percoll is often

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used for assisted reproductive technologies (ARTs), improvement in total motile sperm recovery rates is of critical clinical importance in the treatment of the subfertile male. In fact, the total motile sperm fraction recovered after semen preparation is the single most important criterion used to determine the treatment for male factor patients prior to all forms of assisted reproduction (9). If the number of motile sperm recovered can be raised to 5×10^6 , then the couple may be treated with intrauterine insemination (IUI) rather than more complex and expensive forms of ART such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The current study seeks to determine (a) whether a new, relatively hyperosmotic Percoll gradient, Enhance-S, can improve total motile sperm recovery rates compared with the more commonly used Perception, and (b) whether sperm motion characteristics such as motility and velocity show differences in the sperm recovered by the two types of gradients.

MATERIALS AND METHODS

Sample Collection and Semen Analysis

Semen samples were obtained by masturbation from healthy donors of average reproductive age ($n = 17$) after a 2-day abstinence period. Semen samples were collected into a sterile specimen cup, and the ejaculate was allowed to liquefy at 37°C . A 5- μl sample was loaded onto a 20 μm MicroCell chamber (Conception Technologies, La Jolla, CA) and analyzed on a computer-assisted semen analyzer (Motion Analysis Corporation, Palo Alto, CA; Model VP 110) for sperm count, percentage motility, and curvilinear velocity both before and after preparation. Formal semen analysis was performed according to WHO criteria (10). The remainder of the ejaculate was divided into two equal parts and then prepared with either Enhance-S or Perception. In addition, total motile sperm count was calculated both in the untreated specimen and after the specimen was washed with either of the two Percoll gradients. Total motile sperm count was calculated as follows:

sperm concentration \times ejaculate volume \times percentage motility.

Each of the five technicians who participated in this study processed at least three individual semen samples. The mean intertechnician coefficients of variance were very low: 2.3% for sperm count and 3.1 % for percentage motility.

Percoll Gradient Separation

Discontinuous density gradients (Percoll) were obtained from two sources. The Perception was obtained from Fertility Technologies (Natick, MA), while the Enhance-S was obtained from Irvine Scientific (Santa Ana, CA). Enhance-S is currently distributed by Conception Technologies (La Jolla, CA).

Both Percoll gradients were prepared using 2 ml each of an upper phase (47%) and a lower phase (90%) added into a 15-ml sterile conical centrifuge tube. The upper phase of the Perception had an osmolarity of 301 mOsm/L, while the lower phase had an osmolarity of 310 mOsm/L. In contrast, the upper phase of the Enhance-S had an osmolarity of 317 mOsm/L, while the lower phase had an osmolarity of 361 mOsm/L. Thus, the Enhance-S was a relatively hyperosmolar preparation. An equal portion of liquefied semen sample was gently layered on the upper phase of both gradients. Samples were centrifuged at 1600 rpm for 20 min and the supernatant was discarded. Two milliliters of human tubal fluid (HTF) was added to each sperm suspension and centrifuged for 7 min at 1600 rpm. The supernatants were removed and the sperm were resuspended in 0.5 ml of HTF, and then CASA and formal semen analysis were performed in the manner described earlier.

Statistical Analysis

Data were analyzed for statistical differences in sperm motion parameters between the two treated samples and between treated and untreated samples by repeated-measures of analysis of variance (ANOVA), using the SAS statistical

software package (SAS Institute, Carey, NC). A p value of 0.01 was considered significant.

RESULTS

In the unwashed samples, the ejaculate volume was 1.9 ml (range, 0.6 to 6.5 ml), the sperm concentration $56 \times 10^6/\text{ml}$ (range, 13.9×10^6 to $363 \times 10^6/\text{ml}$), the percentage motility 67% (range, 21% to 90%), and the velocity $38.4 \mu\text{m}/\text{sec}$ (range, 20.6 to $84.2 \mu\text{m}/\text{sec}$). The total motile sperm count in the unwashed specimens was $134.04 \times 10^6 \pm 100.3 \times 10^6$. Compared to the raw specimens, sperm motility improved significantly after preparation with both Perception ($P < 0.0026$) and Enhance-S ($P < 0.0023$). Similarly, velocity improved significantly after washing with Perception and Enhance-S ($P < 0.0001$ for both gradients) compared to the raw specimens (Table 1). However, there was no difference in the degree of improvement in motility and velocity between the two types of Percoll gradients. A significantly higher total motile sperm recovery rate was observed with the Enhance-S gradient than with the Perception gradient ($P < 0.0024$) (Table 1).

Table 1. Comparison of Sperm Characteristics Between the Two Types of Percoll Gradients ^a

Characteristic	Prewash Specimen	Perception Percoll	Enhance-S Percoll
Total motile (10^6)	134.04 ± 100.3	18.9 ± 19.7	30.7 ± 26.0
Motility (%)	66.7 ± 20.6	79.9 ± 17.6	81.1 ± 14.4
Velocity ($\mu\text{m}/\text{msec}$)	38.4 ± 15.8	89.5 ± 11.1	85.6 ± 7.2

^a Results expressed as mean \pm SD.

DISCUSSION

The success or applicability of a sperm preparation method is often assessed by its yield of motile spermatozoa, but other relevant considerations include the labor cost, materials, and equipment, as well as the likely level of contamination with seminal components and the possible exposure of

the spermatozoa to deleterious influences during processing. Compared to other methods of semen processing, the discontinuous Percoll gradient technique has been shown to result in both a higher total yield of sperm and a higher fraction of good-quality, motile sperm (11, 12). Moreover, in vitro studies indicate that the sperm recovered via Percoll filtration techniques may have an enhanced fertilizing capacity (13,14). In our experience, Percoll preparation does not take longer or involve more labor costs than a wash or swim-up technique; indeed, in our laboratory, Percoll preparation is two to three times faster than swim-up methods. The current study indicates that the use of a new, relatively hyperosmotic Percoll gradient, Enhance-S, can improve upon these results by increasing the recovery of total motile sperm.

It is well known that, compared to serum, human ejaculate is relatively hyperosmotic. The osmolarity of the ejaculate ranges from 337 to 457 mOsm/L (15). It is also well established that the spermatozoon is an osmotically active cell and that, when it is placed in a relatively hypotonic environment, water enters the cell until an osmotic equilibrium is reached. Electron microscopic studies have demonstrated that when spermatozoa are filtered through a relatively hypoosmotic conventional Percoll gradient, the sperm swell at both the acrosomal region and the middle portion (16). This swelling increases the volume of the individual spermatozoon; the increased volume decreases the density of the sperm cell and also impairs its motility (17). The decreased density and impaired motility of the sperm processed through a relatively hypoosmotic Percoll gradient result in a lower total motile sperm recovery rate. In contrast, when the sperm pass through a relatively hyperosmotic Percoll gradient, no such increase in swelling takes place. Thus, the sperm have normal density and motility, and the total motile sperm recovery rate is improved. Enhance-S is relatively hyperosmotic compared to Perception. Thus, when the sperm are processed with the Enhance-S, less swelling occurs, the sperm density is nearer normal, and the sperm motility is not impaired,

resulting in an improved total motile sperm recovery rate compared to Percoll.

Our results are in agreement with previous reports of an increase in motile sperm recovery with hyperosmotic Percoll gradients, which also reported an increase in the total motile count recovered with a similar difference in osmolarity (18,19). Moreover, the lack of any difference in motility and velocity between sperm recovered via the two gradients indicates that the improvement in total motile sperm recovery noted with Enhance-S is not being achieved by the recovery of sluggish, relatively poorly motile sperm but, rather, by the recovery of good-quality sperm. The lack of any significant difference in the percentage motility between the two preps stems from the fact that the additional sperm recovered via Enhance-S are largely motile ones; thus, while the total motile count increases, the percentage of motile sperm does not.

This Percoll gradient may be especially useful in processing oligospermic specimens for ART procedures requiring relatively high number of sperm, such as IUI. In general, there is an increase in the pregnancy rate with increasing numbers of motile sperm inseminated (20,21). Enabling the subfertile couple to achieve pregnancy via IUI can result in a significant savings for the couple. It is estimated that IUI can cost three to five times less than more complex methods of ART (22). Moreover, processing semen via the use of a hyperosmotic Percoll gradient should theoretically improve the fertilization rate obtained with assisted reproduction. According to Jeyendran et al., only

those sperm with normal function and perfect membrane integrity can swell in a hypoosmotic environment (17). Thus, sperm that are lost during recovery with a relatively hypoosmotic Percoll gradient are the very sperm most likely to have a good fertilizing capacity, namely, sperm with normal function and perfect membrane integrity. In contrast, these sperm should not be subject to swelling stresses in a relatively hyperosmotic Percoll gradient, and thus, they should be recovered for use in conjunction with ARTs. A prospective study comparing pregnancy rates obtained from ARTs using sperm recovered with either a relatively hypoosmotic or a hyperosmotic Percoll gradient might answer this question.

CONCLUSIONS

In conclusion, our data show that the use of a hyperosmotic Percoll gradient significantly improves the recovery of total motile sperm. The difference in total motile sperm recovery between the two types of Percoll gradients may actually result from the loss of the very sperm most likely to have good fertilizing capacity, and thus, the use of hyperosmotic Percoll gradient may ultimately enable the subfertile couple to pursue less costly methods of assisted reproduction.

ACKNOWLEDGEMENTS

The authors thank Debbie Garlak, M. T., Cheryl Fitzugh, M. T., Suzanne Kohn, M. T., and Lora Cordek, M. T., for their technical assistance and Jar-Chi Lee, M.S., for statistical analysis.

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