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Comparison of Six Density Gradient Media for Selection of Cryopreserved Donor Spermatozoa

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ABSTRACT: The aim of our study was to evaluate the efficiency of 4 density gradient media for motile cryopreserved spermatozoa selection to Percoll (Kabi Pharmacia, Uppsala, Sweden) and to Puresperm (J.C.D. International Laboratory, L'Aigle, France). Puresperm was the new medium chosen in our laboratory in 1996 as the substitute for Percoll. The solutions tested were 3 colloidal silane-coated silica particle media (Isolate, SpermGrad-100, Sil-Select Plus) and iodixanol (Optiprep). Semen parameters analyzed after selection were concentration, motility, and morphology. Semen parameters after Puresperm gradient had similar values compared to Percoll. Optiprep was less efficient with a poor concentration. Isolate had a comparatively better concentration, but the capacity of selection was not satisfactory. SpermGrad-100 and Sil-Select Plus were less effective than Puresperm. In conclusion, Puresperm could be considered a better alternative to Percoll for cryopreserved spermatozoa migration.

Key words: Sperm separation, silane-coated silica particles, iodixanol, donor semen.

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An efficient sperm preparation technique for assisted reproduction requires the capability to accumulate the largest number of morphologically normal and motile spermatozoa in a relatively small volume. In addition, this preparation must be free of seminal plasma, leukocytes, bacteria, and other debris. Percoll gradient density (Kabi Pharmacia, Uppsala, Sweden) has been used extensively for spermatozoa selection. Several studies have proved Percoll's effectiveness (Iizuka et al, 1988; Mathieu et al, 1988; Punjabi et al, 1990; Claassens et al, 1996) and its comparatively better selection compared with simple washing or swim-up preparation (Berger et al, 1985; McClure et al, 1989; Bongso et al, 1993; Moohan and Lindsay, 1995). Good clinical pregnancy rates were obtained after Percoll migration (Pardo et al, 1988; Sato et al, 1990), higher compared with swim-up preparation (Guérin et al, 1989). However, concerns have been raised regarding Percoll's safety, and its use in assisted reproductive technologies has been withdrawn since 1996. On this date, we substituted Percoll with Puresperm (J.C.D. International Laboratory, L'Aigle, France), and Percoll was used only for the tests.

Different gradient media have been proposed by other manufacturers, such as Iodixanol (Harrison, 1997), or several silane-coated silica particle colloid solutions (Perez et al, 1997).

The medium used for cryopreserved spermatozoa selection must be highly accurate since cryopreservation causes a significant decrease in spermatozoa motility (Sharma and Agarwal, 1996). The aim of our study was to compare 3 colloidal silane-coated silica particle solutions (Isolate, SpermGrad-100, Sil-Select Plus) and an iodixanol solution (Optiprep) with Puresperm and Percoll for selection of motile spermatozoa from cryopreserved donor semen samples to evaluate the most effective solution.

Materials and Methods

Semen Samples

Specimens from 12 volunteer donors were collected by masturbation in sterile containers after 3 to 5 days of sexual abstinence. After 20 minutes of liquefaction at 37°C, conventional semen analysis for concentration and motility was performed according to World Health Organization criteria (World Health Organization, 1999). Then, semen samples were diluted in a cryoprotector (Ackerman medium) and automatically frozen in straws with a Minicool LC 40 (Air Liquide Santé, France). Straws were stored in liquid nitrogen at −196°C. Two straws were thawed at 37°C for 5 minutes for each gradient centrifugation technique.

Density Gradient Preparation

All gradient solutions were diluted with Ferticult medium (J.C.D. International Laboratory). The different gradients were

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prepared taking into account the recommendations of the manufacturers.

For Percoll (Kabi Pharmacia) and Sil-Select Plus (Fertipro, Beernem, Belgium) solutions, a 2-layer gradient was prepared with 45% and 90% fractions. For Puresperm (J.C.D. International Laboratory), Isolate (Clinisciences, Mont-Rouge, France), and SpermGrad-100 (IVF Science Scandinavia, Gothenburg, Sweden), the solutions were diluted to obtain 70% and 90% fractions. The thawed semen sample (0.5 mL) was layered on the top of the gradients.

Optiprep gradient (Nycomed Pharma, Oslo, Norway) was prepared with 45% and 28% fractions. Semen was diluted with 0.5 mL Ferticult medium and pipetted into the bottom of a 10-mL tube containing 1 mL of Optiprep stock solution. Two milliliters of 45% fraction were layered above the semen, and 2 ml of 28% fraction were layered on the top of the first fraction.

The 6 gradients were centrifuged at 150 × *g* for 20 minutes. Then the 90% fraction was washed by centrifugation with Ferticult medium at 350 × *g* for 10 minutes for Percoll, Puresperm, Isolate, SpermGrad-100, and Sil-Select Plus gradients. The interface between the 45% and 28% fractions of the Optiprep gradient containing motile spermatozoa was centrifuged with Ferticult medium at 350 × *g* for 10 minutes. The final pellet of the 6 density gradients was resuspended in 0.5 mL of washing medium and incubated at 37°C with 5% CO_2.

Data and Statistical Analysis

The parameters analyzed on the final suspension were concentration (million/mL [M/mL]), progressive motility (rapid plus slow progressive motility [a + b]), and morphology. Morphology evaluation was performed on slides stained by a Shorr method (World Health Organization, 1999) using Kruger and David modified criteria (Jouannet et al, 1988; Kruger et al, 1988).

Results were expressed as mean plus or minus standard error of mean. The Friedman test was used for total comparison using the logicial Statview for Windows (SAS Institute Inc, Cary, NC). When the Friedman test revealed a statistical difference ($P < .05$), the Wilcoxon test with Bonferroni correction was used to compare the media with Puresperm and Percoll.

Results

Results (mean ± SEM) are shown in the Table. All the semen parameters explored were significantly different between the 6 media (Percoll, Puresperm, Isolate, Optiprep, SpermGrad-100, Sil-Select Plus) with $P < .0001$ for concentration, $P = .0023$ for motility, and $P = .002$ for morphology.

Even if the semen parameters were slightly improved for Percoll, no significant statistical difference was observed between Percoll and Puresperm.

Spermatozoa concentration after selection by Puresperm, SpermGrad-100, and Sil-Select Plus had similar values. The optimal concentration was obtained with Isolate ($P < .05$) compared with Puresperm, then with Percoll. Optiprep had the lowest value ($P < .05$) compared with Percoll and Puresperm.

Comparison between the values of the semen parameters analyzed for the 6 gradient techniques (values are mean ± SEM)

Gradients	Concentration, M/mL*	Percent† Progressive Motility (a + b)	Percent† Normal Morphology
Percoll	10.12 ± 2.3[a]	46.7 ± 4[e]	69.3 ± 2.5[l]
Puresperm	7.33 ± 1.08[b]	45 ± 3.8[f]	66.1 ± 2.2[j]
Isolate	14.42 ± 1.58[c]	29.6 ± 3.6[g]	63.2 ± 2.7
Optiprep	2.61 ± 0.48[d]	42.1 ± 4.5	52.5 ± 2.7[k]
SpermGrad-100	6.92 ± 0.9	32.1 ± 2.9[h]	60.3 ± 2.5
Sil-Select Plus	6.58 ± 0.79	35 ± 2.6	65.3 ± 3

* a–d, $P = .0198$; b–c, $P = .0486$; b–d, $P = .0297$.
† e–g, $P = .0297$; f–h, $P = .0396$.
‡ i–k, $P = .0423$; j–k, $P = .0261$.

Percoll, Puresperm, and Optiprep gradients led to the highest progressive motility (46%, 45%, and 42%, respectively), whereas Isolate had a significantly lower frequency compared with Percoll ($P < .05$) and SpermGrad-100 compared with Puresperm ($P < .05$).

The selection of morphologically normal spermatozoa was significantly lower for Optiprep compared with Percoll and Puresperm ($P < .05$).

Discussion

Percoll has routinely been used to select motile spermatozoa in assisted reproductive techniques. In 1996, it was removed from clinical human use because of possible endotoxin contamination. The aim of our study was to compare 4 gradients with Percoll and with the new gradient solution chosen in our laboratory, Puresperm (colloidal silane-coated silica particles), in selection of frozen-thawed ejaculated spermatozoa to identify the best substitute. Twelve cryopreserved donor semen samples were tested in this experiment. The 4 gradient media were 3 solutions with colloidal silane-coated silica particles (Isolate, SpermGrad-100, and Sil-Select Plus) and an Iodixanol solution (Optiprep).

Our results showed that Puresperm had similar values to Percoll with no significant difference for the parameters explored. These data are in agreement with other studies (Centola et al, 1998; Claassens et al, 1998). However, in one study, Percoll was found to be significantly superior to Puresperm (Chen and Bongso, 1999), but the authors concluded that Puresperm produced a good sperm separation and was an attractive alternative. Söderlund and Lundin (2000) found no difference in sperm motility recovery when they compared 4-layer gradients of Percoll and Puresperm. However, they observed a significant de-

crease of motility when a 2-layer gradient of Puresperm was used.

Isolate presented the optimal concentration. However, Isolate's separation of motile and immotile spermatozoa was not effective: progressive motility was significantly decreased compared with Percoll. Other cells could be selected with immotile spermatozoa like leukocytes, immature sperm cells, and microorganisms. These elements and the lower motility could affect spermatozoa fertilization ability and could be associated with lower pregnancy rates following intrauterine inseminations (Keel and Webster, 1989; Clarke et al, 1997). In contrast, Makkar et al (1999) obtained an improved sperm recovery rate with Isolate compared with Percoll. In other studies, no difference was observed between Isolate and Percoll (Claassens et al, 1998; Sharma et al, 1999). However, since semen samples explored were fresh, the efficiency of Isolate was probably decreased on frozen-thawed spermatozoa.

Optiprep was the less effective gradient, with significantly lower concentration and normal morphology compared with Percoll and Puresperm in agreement with the reports of Claassens et al (1998). In contrast, no difference was reported between Percoll and Optiprep for the same parameters in fresh semen (Smith et al, 1997) or improved results for Optiprep (Andersen and Grinsted, 1997).

SpermGrad-100 and Sil-Select Plus had similar results, but they were less effective than Percoll and Puresperm regarding progressive motility, with a significant difference for SpermGrad-100.

In conclusion, Puresperm, with similar sperm parameters to Percoll, could be considered a suitable substitution medium. Optiprep had a poor spermatozoa concentration. Isolate had the optimal concentration, but its selection was not satisfactory with a large number of immotile spermatozoa. SpermGrad-100 and Sil-Select Plus had medium values; however, Puresperm had the comparatively better parameters. Therefore, our results suggest Puresperm could be considered for the selection of fresh and frozen-thawed spermatozoa in assisted reproductive techniques.

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