ANDROLOGY

Iodixanol as a Density Gradient Medium for the Isolation of Motile Spermatozoa

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Purpose: The purpose of this study was to establish concentrations of Iodixanol which could be used in a similar manner to the widely used Mini-Percoll technique for the separation of motile spermatozoa.

Methods: Various density gradient combinations of Iodixanol were compared for the isolation of motile spermatozoa from cryopreserved donor semen. The toxicity of Iodixanol was tested by its effect on the growth of two-cell mouse embryos and fresh sperm survival.

Results: The best sperm recovery (32%) came from the pellet of sperm passing through a discontinuous gradient of 25% over 40% OptiPrep after 20 min of centrifugation at 400 g. There was no evidence of toxicity to mouse embryo growth or sperm survival.

Conclusions: Iodixanol (OptiPrep; Nycomed) provides a satisfactory alternative for the efficient separation of motile spermatozoa for ART procedures.

KEY WORDS: sperm separation; density gradient; Iodixanol.

INTRODUCTION

The isolation of the best motile sperm from a semen sample by centrifugation through a continuous (1) or discontinuous (2) density gradient medium, most commonly Percoll (Pharmacia), has been steadily refined to a point where its simplicity (3,4) makes it the preferred method for preparing sperm for assisted reproduction treatments. Apart from separating the best motile sperm, the method reduces bacterial contamination of

the final preparation (5). In male-factor infertility cases it reportedly improves oocyte fertilization (4) and sperm performance in the hamster ova sperm penetration assay (3) over the swim-up technique. With Percoll becoming unavailable for use in human assisted reproductive technology (ART), an acceptable alternative is required. The nonionic iodinated compound Nycodenz (Nycomed), developed as an X-ray contrast medium, has been advocated for this use in the past and was claimed to perform better than Percoll (7). More recently a dimeric form of Nycodenz, Iodixanol (OptiPrep; Nycomed), has been promoted as a superior density gradient medium with the added advantage of its having passed strict clinical testing for its use in X-ray studies. Flotation separation using discontinuous gradients has been shown to work for human sperm (8), but in samples with low numbers of spermatozoa, their harvesting from an interface can prove difficult. This study reports the use of OptiPrep to separate motile spermatozoa by centrifugation through two layers of medium into a pellet at the bottom of a conical tube, where it is simpler to recover them all in a manner similar to that in which Percoll has been commonly used (3).

MATERIALS AND METHODS

Sperm Separation

Stock OptiPrep was prepared by mixing 9 parts of Iodixanol (OptiPrep; Nycomed) with 1 part of 10× concentrated Ham's F-10 medium (Sigma Chemical Co.). To produce the required density solutions, this

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stock solution was diluted with human tubal fluid (HTF) medium (9). Discontinuous gradients were prepared in 15-ml conical-based tissue culture tubes (Falcon 2095, Becton Dickinson) with two layers of 1.0-ml aliquots selected from 25, 35, 40, 45, 50, 60, 70, 80, and 90% dilutions of stock OptiPrep.

The test semen was 0.5-ml aliquots of cryopreserved donor semen containing 25 million progressively motile sperm. The thawed semen was brought to 37°C before being gently layered on top of the upper layer of OptiPrep. The tubes were centrifuged at 400g for 20 min (and in one experiment for 15 min) in a centrifuge with swinging bucket rotor. The resulting pellet was gently aspirated, centrifugally washed once in 5 ml of HTF medium, and suspended in 1 ml of HTF medium for sperm counting. Preparations were repeated in triplicate and the mean yield was calculated.

Toxicity Testing

A 25- μ l drop of each of 25, 40, and 100% OptiPrep was layered onto the bottom of wells containing 25 F₁ CBA/C57 two-cell mouse embryos in 1 ml of HTF, which were then incubated for 72 hr at 37°C in a 5% carbon dioxide environment before being evaluated for growth to blastocyst. The Optiprep formed a fine layer on the bottom of the wells and the embryos were seen to float upon it. Fresh normal sperm separated by the 30/45% and 25/40% gradients were incubated for 72 hr in HTF medium at 37°C in a 5% carbon dioxide environment before evaluation for maintenance of progressive motility.

RESULTS

Sperm recoveries from the various OptiPrep gradients are shown in Table I. It can be seen that the best sperm recovery (32%) came from the pellet of sperm

passing through a discontinuous gradient of 25% over 40% OptiPrep after 20 min of centrifugation at 400g. Shortening of the centrifugation time to 15 min resulted in reduced sperm recovery.

Fresh normal sperm separated by the 30/45% and 25/40% gradients and incubated for 72 hr in standard culture conditions demonstrated greater than 80% maintenance of motility. Two-cell F₁ CBA/C57 mouse embryos grown in 1 ml of HTF medium with an added drop of each of 25, 40, and 100% OptiPrep showed 86, 85, and 82% growth through to blastocysts when incubated for 72 hr under standard culture conditions.

DISCUSSION

The modern assisted reproductive technologies have a need for a rapid, simple, and reliable method for the separation of the most strongly motile sperm. Density gradient centrifugation provides the most reliable means of achieving this, and Percoll has been the most widely used medium for the past decade. With its restriction from human use, an alternative is required. The study reported here suggests that Iodixanol (OptiPrep; Nycomed) is an efficient and safe alternative. The approach of centrifugation to a pellet in a conical-based test tube as advocated here allows simpler and more efficient harvesting of the spermatozoa than the flotation separation approach.

Nonionic iodinated compounds have presented a potentially safe alternative for sperm separation since their introduction as Nycodenz, which was found to perform equally with Percoll in 1988 (7). With Nycodenz, 12 min of centrifugation at 350g using a discontinuous gradient ranging from 35 to 100% gave a satisfactory result. OptiPrep, being essentially a dimer of Nycodenz, is denser and therefore can be used at lower concentrations. This provides an added safety margin should there be any concerns of toxicity. The compound was developed for X-ray contrast use and has been found to show no toxicity in clinical trials (10). Embryo and sperm toxicity studies performed as part of the study reported here show no toxic or adverse effects.

Concentrations lower than 25 over 40% were not tried for handling reasons. Less than 25% in the upper layer would make the layering of raw semen on top of it too difficult and a differential of less than 15% between layers would allow more rapid miscibility of the layers.

Table I. Sperm Recoveries from Various OptiPrep Gradients

Gradient (% OptiPrep)	Centrifugation time (min)	Mean % sperm recovery	Range of % sperm recovery
45/90	20	Nil	
30/70	20	Nil	
30/60	20	7.7	7.2–8.4
30/50	20	21.9	20.4–24.0
30/45	20	28.1	27.6–28.8
25/40	20	32.1	30.0–34.0
25/40	15	24.8	24.0–25.6

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