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Sperm Separation Technique Using Iodixanol Compare to Percoll Gradient

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ABSTRACT

Objective To evaluate the use of iodixanol for the separation of sperm motile fraction and the effect on sperm morphology comparing to Percoll gradient.

Design Experimental study.

Setting Research center, Ramathibodi Hospital.

Methods Thirty semen samples from infertile patients were studied. The initial evaluation of sperm count and morphology was performed. The samples were then equally aliquoted into 2 parts and were separately prepared by discontinuous gradient using Percoll and iodixanol. The yielded sperm from the two preparations were compared under the following parameters: motile and non-motile sperm count, progressive motion and percentage of normal morphology.

Results Concentrations of motile sperm after sperm preparation by both Percoll and iodixanol were comparable (17.4±11.7 VS 16.3±14.0 $x10^6$/ml). Non-motile sperm count from Percoll and iodixanol were lower than the initial sperm (6.2±9.9, 18.1±19.1, and 24.2±15.9 $x10^6$/ml, respectively). Iodixanol produced higher non-motile sperm concentration compared to Percoll. Both gradient materials gave comparable improvement of progressive movement from 2.2±0.3 in initial sperm to 2.9±0.2 by Percoll and 2.8±0.2 by iodixanol. Percoll gradient gave a significant improvement of the percentage of normal sperm morphology from 14.1±3.3 % in initial sperm to 16.8±5.3 %, while there was no change after iodixanol gradient. Percentages of head and tail defects were not changed after preparation by either gradient but midpiece defect was significantly decreased from 11.1±3.6 % to 7.8±4.3 % after Percoll process.

Conclusion Although motile sperm yielded by Percoll and iodixanol were comparable, Percoll gradient gave a better percent motility because it could separate more non-motile sperm. Percoll gradient also gave a better percentage of morphologically normal spermatozoa compared to iodixanol gradient. This new medium could replace Percoll if some preparation procedures are adjusted.

Key words: iodixanol, Percoll, sperm separation, density gradient centrifugation

Smith et al.[8] demonstrated that by modifying the iodixanol gradient volumes, the centrifugal force, and the duration of the centrifugation can dramatically improve the yield of motile and morphologically normal spermatozoa.

While the motile sperm counts were comparable, the percent motility was not improved following iodixanol density gradient centrifugation but significantly improved following Percoll gradient centrifugation. The finding indicates that separation of the non-motile fraction by using Percoll gradient is better than by using iodixanol gradient. This can be explained by the lower density of the medium and the lower differential gradient between upper and lower layers of the Ixaprep, compared to 40%, 90% Percoll gradient. The lower layer of IxaPrep had a density of 1.09 g/ml, which was less than that of 90% Percoll (1.12 g/ml). The upper layer of IxaPrep had a density of 1.05 g/ml, which was a little bit less than that of 40% Percoll (1.052 g/ml). The lower density of IxaPrep might be responsible for the passage of both lower motile and non-motile spermatozoa into the resultant pellet. The low differential gradient of IxaPrep made an inefficient separation of non-motile spermatozoa at the interface of the upper and lower layer.

Although there are evidences that preparation of sperm by differential gradient centrifugation can select better population of morphologically normal sperm compared to other method,[5,14] we did not find any improvement of normal sperm morphology by using iodixanol. However, we found that by using Percoll gradient the morphologically normal sperm was significantly improved and the improvement in the percentage of midpiece defects was the only contributing factor in the overall improvement in the normal sperm morphology. Prakash et al.[5] also found that the percentage of sperm with normal head morphology was not improved by using Percoll gradient centrifugation but midpiece and tail morphology were improved. As it is believed that differential gradient centrifugation separates sperm on the basis of density, the sperm orient themselves down into the gradient solution as long as their specific gravity is higher than that of the surrounding medium. The sperm with midpiece defects loss its specific density to go down into the lower layer so the percentage of midpiece defects is decrease. In contrast, the lower density of iodixanol both upper and lower layer together with the lower differential gradient between the layers compared to Percoll gradient might be the cause of failure to separate the morphologically abnormal spermatozoa.

Although these data demonstrated no advantage from the use of iodixanol over Percoll for the separation of spermatozoa with respect to sperm recovery, sperm motility and sperm morphology, its safety has been confirmed through a series of clinical trials.[15-17] As iodixanol is a dimeric form of iohexol, the desired densities can be achieved by lower amount of the gradient substance. This factor may give an additional safety compared to other mediums. The better sperm recovery, motility and morphologically normal spermatozoa of the yield pellet prepared by iodixanol gradient may be achieved by increasing the density of the upper and lower layers and adjust the appropriate differential gradient between the layers which need further investigation.

Table 1. Concentration of motile sperm, non-motile sperm concentration, progressive motion and percent of normal sperm morphology from initial sample and after sperm preparation by Percoll and iodixanol gradient centrifugation. (value presented as mean ± SD)

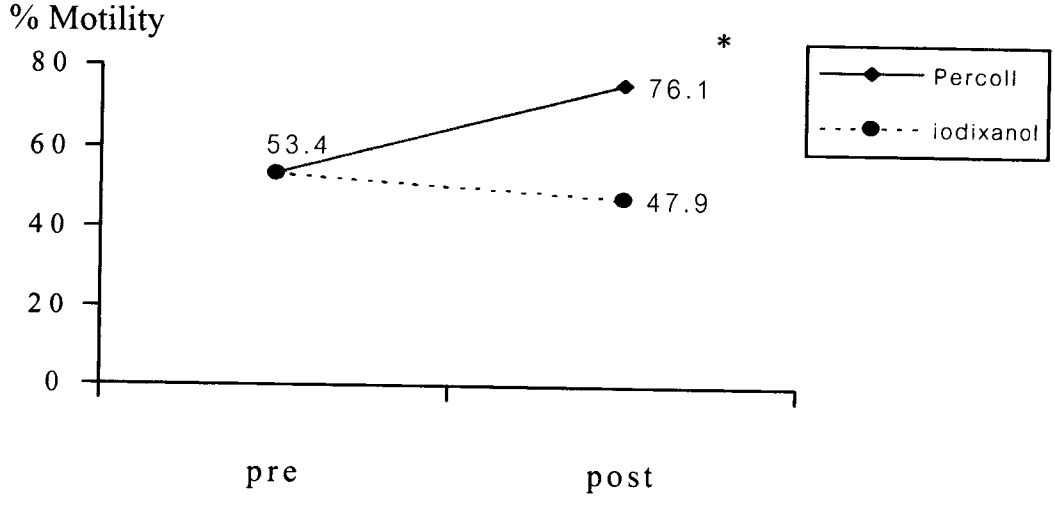
	motile ($x10^6$/ml)	non-motile ($x10^6$/ml)	Progression	Morphology (%)
Initial sample	28.3±17.1 [a]	24.2±15.9 [a]	2.2±0.3 [a]	14.1±3.3 [a]
Post-Percoll	17.4±11.7 [b]	6.2±9.9 [b]	2.9±0.2 [b]	16.8±5.3 [b]
Post-iodixanol	16.3±14.0 [b]	18.1±19.1 [c]	2.8±0.2 [b]	12.5±4.2 [a]

Different superscription in the corresponding parameter means $P<0.05$. The same superscription in the corresponding parameter means there is no statistical significance.

Table 2. Head, midpiece and tail abnormalities in initial sperm and after sperm preparation by Percoll and iodixanol gradient centrifugation. (value presented as mean ± SD)

	Type of abnormal morphology (%)		
	Head	Midpiece	Tail
Initial sample	63.7±6.5 [a]	11.1±3.6 [a]	11.0±4.2 [a]
Post-Percoll	65.1±9.1 [a]	7.8±4.3 [b]	10.5±3.7 [a]
Post-iodixanol	66.2±9.0 [a]	10.6±4.2 [a]	11.0±5.2 [a]

Different superscription in the corresponding parameter means $P<0.05$. The same superscription in the corresponding parameter means there is no statistical significance.



* $p<0.05$, compare to pre-preparation

Fig. 1. Percent motility of pre-preparation and post-preparation by Percoll and iodixanol gradient centrifugation.

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