A New Percoll Method for Motile Spermatozoa Selection In Asthenozoospermic and Leucocytospermic Samples*

ASTENOSPERMİK VE LÖKOSİTOSPERMİK ÖRNEKLERDE MOTİL SPERMA TOZOALARI AYIRT ETMEK İÇİN YENİ BİR PERCOLL METODU

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SUMMARY

Objective; Three techniques for the separation of motile spermatozoa were compared.

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Materials and Methods: Ejaculates were collected from asthenozoospermic and leucocytospermic patients. The samples were divided into three allquots of equal volume. Each of them was processed by the swim up migration, centrifugation on a discontinuous percoll gradient and centrifugation on a 40% of percoll. Their respective effects on sperm motility were analyzed.

Findings: In three groups, there was no difference in any of the motion parameters between the swim up or discontinuous difference in motility and in the number of norma! forms was observed after 40% percent percoll in 400 rpm centrifugation.

Results: We conclude that, with this modification the technique of 40% percent of percoll in 400 rpm used for the separation of human spermatozoa is strongly advised especially in asthenozoospermic and leucocytospermic samples.

Key Words: Human spermatozoa, Asthenozoospermia, Leucocytospermia, Percoll, Swim up

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ÖZET

Amaç: Motil spermleri ayırmak için üç farklı yıkama tekniği karşılaştırıldı.

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Materyal ve Metod: Astenospermik ve lökositospermik semen örnekleri laboratuarda 3 eşit miktara ayrıldı. Bu örnekler swim up, aralıklı percoll gradient ve %40'lık percoll ile yıkandı.

Bulgular: Bir saatlik inkübasyon zamanından sonra swim up ve aralıklı percoll gradient tekniklerinin spermin hareket parametrelerine etkisi arasında fark olmadığı, buna karşılık 400 rpm. sentrifugasyonda yapılan %40'lık percoll tekniğinin motiliteyi anlamlı olarak arttırdığı tespit edildi.

Sonuç: Buna göre %40'lık percoll tekniğinin özellikle astenospermik ve lökositospermik örneklerde motil spermleri ayırt etmek için kullanılması tavsiye edilir.

Anahtar Kelimeler: Spermatozoa, Astenospermlum, Lökositospermium, Percoll, Swim up

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The progressive motility is considered to be the most important in relation to the rate of fertilization (1). The techniques of swim up migration and discontinous percoll gradient are routinely used by most teams to select motile gametes in cases of asthenozoospermia and leucocytospermia (2). Alternative methods to swim up techniques have been suggested for the isolation of motile spermatozoa from poor semen samples. The most succesful of these involve the passage of spermatozoa through concentration gradients of high density media such as albumin or percoll (3,4). Sperm selection on percoll gradients has been reported to yield up 60% of the motile, morphologically normal spermatozoa from a normal semen sample. However the recovery of normal motile spermatozoa from Oligozoospermie and asthenozoospermic samples was not found to be as great nor as consistent as for normal samples.

In this study we have compared the effects of three treatments - Swim up migration, centrifugation on a discontinous percoll gradient and centrifugation on 40% of percoll In 400 rpm- on sperm motility two

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Tablet. Leucocytospermic samples
Tablo 1. Lökositospermik örnekler

	Concent-ration (million/ml)	Total cell (million)	Motility (%)	Leu-cocytes (million/ml)	Rocovery of motile cells (X10^6) P.G.	40P	S.U.	% Recovery motile cells P.G.	40P	S.U	Final Leucocytes (million/ml) P.G.	40P	S.U
1-	60	180	27	9	3.280	7.170	6.336	6.74	14.75	13.2	1	0.98	1
2-	49	245	38	7	12.890	38.000	33.400	13.7	41	36	0.65	0.5	0.35
3-	70	140	25	6	15.150	25.000	14.000	43.2	71	40	2	1.5	0.75
4-	40	240	45	4	10.060	30.000	19.440	9.3	28	18	0.5	0.8	0.6
5-	39	78	50	2.5	8.490	30.000	17589	21.76	76	45	0.7	0.8	0.5
6-	23	69	30	4	4.800	15.000	10.184	23.18	72.4	49.2	0.5	0.4	0.5
7-	14 -	56	30	3	1.570	8.000	4.700	'9.34	47.6	28.2	0.75	0.6	0.3
8-	15	60	18	9	540	5.000	3.823	5	46.2	35.4	0.8	0.8	1
			32.87+10.69				16.52 ± 12.66		49.61 ± 22.18		33.12 ± 12.59		

P.G: Percoll Gradient
40 P: 40a Percoll
S.U: Swim UP
Wilcoxon-t; s.d.: 8, p<0.01

groups of men. The first group consisted of leucocytospermic and the second group consisted of asthenozoospermic patients.

MATERIAL AND METHODS

Ejaculates (n:16) were collected by masturbation after at least 48 hours of sexual abstinence from asthenozoospermic and leucocytospermic patients consulting for infertility problems. The semen was allowed to liquefy at 37 C for 20 to 30 minutes and the samples were divided into three aliquots of equal volume. First aliquot was processed by the swim up method and the second aliquot was processed by the discontinous percoll gradient method and the third aliquot was processed by the 40% of percoll method.

Swim up; The semen sample was washed with 4 ml of Earles solution (Gibco) supplemented with 3 mg/ml of bovine serum albumin (Sigma) by centrigugation for 10 minutes at 1500 rpm. After discarding the supernatant, the pellet was ressuspensed in 1.5 ml of EBSS and centrifuged for 5 minute at 1500 rpm. The supernatant was carefully removed and the pellet was covered with medium supplemented with 26 mg/ml BSA. At the end of 1 hour, the upper fraction, containing the motile cells was collected in 0.5 ml of EBSS and the concentration of spermatozoa was determined and adjusted to $1x10^7$ spermatozoa/ml. The tubes were incubated at 37 C in an atmosphere of 5% C02 in air. All the media were filtered through a filter of 0.22 um pore size before use. The percentage of motile spermatozoa was determined at the beining of incubation and after 1 hour incubation.

Percoll gradient; Stock percoll solution was prepared by diluting 9 parts of percoll with 1 part of (10X) tyrode medium (Eurobio, Paris). This stock solution was designated 100% stock percoll solution. The pH and osmolarity were 7.5 and 330 mOsm/lt respectively. Solutions off different percentages of STP. (40%, 60%, 70%, 80% and 90%) were made by combining 100% with 10X Tyrode with 100 IU/ml and 100 (jg/ml of penicillin -streptomycin added respectively. A discontinous one step gradient was carried out by placing 0.5 ml of each solution in a 100 ml sterile conical plastic tube (Falcon), starting with the most concentrated on the top. Freshly collected semen (0.5 to 2 ml) was loaded gently on top and centrifuged at 1500 rpm. for 20 minutes at 22 C. The most concentrated fraction was collected from the buttom of the tube by removing the liquid just at the surface and the remainder was then washed once in 6 ml of EBSS and centrifuged at 1500 rpm for 10 minutes at 22 C. Five tenths mililiter of EBSS was mixed with 0.3% (final concentration) of human albumin for IUl and this solution was carefully added to the pellet. Sperm samples before and after seperation by gradients and swim up were analyzed. Sperm were counted using neubeaer hemocytometer.

40% percoll; The semen (250 ul) was layered on top of 40% of percoll (Isotonic percoll+Tyrodes) and centrifuged at 400 rpm for 10 minutes at 22 C. Pellet was collected by a sterile pasteur pipet and washed with 4 ml of Earles solution supplemented with 3 mg/ml BSA. Samples were analyzed after 1 hour incubation, counted using neubeauer hemocytometer. Wilcoxon t-test was used for statisticla analysis.

Table 2. Asthenospermie Samples
Tablo 2. Astenospermik örnekler

	Concent-ration (million/ml)	Total cell (million)	Motility (%)	Rocovery of motile cells (x10°) P.G.	40P	S.U.	% Recovery motile ceils P.G.	40P	S.U
1-	45	112.5	20	4.657	11.070	3.600	20.7	49.2	16
2-	36	72	15	2.149	7.560	1.663	19.9	70	15.4
3-	24	72	15	2.386	3.780	1.852	22.1	35	17.2
4-	19	76	20	3.313	10.336	2.000	21.8	68	13.1
5-	20	80	19	3.800	8.360	2.584	25	55	17
6-	40	80	20	3.520	7.520	2.688	22	47	16.8
7-	29	58	25	4.060	6.699	2.813	28	46.2	19.9
8.	24	48	**4**	345	576	278	18	30	14.5
				17.25±6.22			22.18	50.05	16.23
							3.09	14.13	2.03

Wilcoxon-t; s.d: 8, p<0.01

RESULTS

The concentration of motile spermatozoa and the percentage of spermatozoa recovered were higher when the 40% of percoll method was used than when the swim up method and percoll gradient method was used for selection (Table 1 and 2).

DISCUSSION

The present results show that the 40% percoll method produced the recovery of a higher number of both total and motile spermatozoa than swim up and discontinous percoll gradient method. Various procedures including swim up, washing and density gradients have been used recently in attemps to improve sperm motility for IVF or intrauterine insemination treatments (5). Because of poor results whwn swim up or simple washing methods were applied to male factor semen, we have been evaluating the use of density gradient procedurs. Percoll has been used perviously tor density gradient centrifugation of spermatozoa and several reports note good yields and final motilities (6). In tnis study we choose to compare the efficacy of motile sperm selection by discontinous percoll with 40 percoll. A grear number of motile spermatozoa were harvested from 40% percoll gradients than from discontinous percoll and swim up procedures.

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