

A clinical study comparing PureSperm and SpermFilter for density gradient separation of human spermatozoa in assisted reproduction

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Objective. To compare a new density gradient medium, SpermFilter, for purifying spermatozoa in assisted reproduction with the more established medium, PureSperm.

Design. Part 1, a multicenter study on 225 semen samples purified using either PureSperm (115 semen samples) or SpermFilter (110 semen samples). Part 2, a retrospective, single center study on a total of 898 assisted reproductive cycles (245 insemination cycles using husband semen, 58 insemination cycles using donor semen and 595 *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles).

Setting. Part 1, three fertility clinics in Denmark (two university-affiliated fertility clinics and one private clinic). Part 2, one university-affiliated fertility clinic in Denmark.

Main outcome parameters. Part 1, purity of purified spermatozoa (% motile), motility index and recovery of motile spermatozoa. Part 2, malformation and baby take-home rates (insemination cycles), fertilization, cleavage, implantation, malformation and baby take-home rates (IVF/ICSI cycles).

Results. No statistical differences were observed in any of the parameters investigated.

Conclusion. SpermFilter is a valid alternative to PureSperm in assisted reproduction technology (ART).

Key words: SpermFilter; PureSperm; semen processing; spermatozoa purification

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Discontinuous density gradient separation (DDGS) of human spermatozoa using silica-based media has been shown to be superior to other methods for modern assisted reproductive techniques: (i) DDGS is more efficient than

swim-up for purification of motile spermatozoa in terms of better recovery rate of motile spermatozoa and higher percentages of motile spermatozoa with good fertilization capability (1–4). (ii) Motile spermatozoa are quickly removed from the seminal plasma, somatic cells and dead and aberrant spermatozoa. Purified spermatozoa are thereby protected from potential stress of reactive oxygen species, which have been shown to reduce the fertilizing capability (5,6). (iii) DDGS reduces and can in combination with strictly aseptic techniques completely abolish bacterial contaminations of spermatozoa from

Abbreviations:

ART: assisted reproductive technology; DDGS: discontinuous density gradient separation; FDA: Food and Drug Administration; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IUI-D: intrauterine insemination with donor semen; IUI-H: intrauterine insemination with husband semen; IVF: *in vitro* fertilization; NS, not significant; PVP: polyvinylpyrrolidone.

bacteriospermia semen samples (7,8). (iv) DDGS eliminates human immunodeficiency virus (HIV) in purified sperm samples from men with HIV infections (9–11). (v) DDGS is superior to swim-up by separating out the spermatozoa with nicked and poorly condensed DNA (12).

Initially, a polyvinylpyrrolidone (PVP)-coated silica medium (Percoll, Pharmacia Biotech AB, Uppsala, Sweden) was used as the density medium of choice, but in 1996, Percoll was withdrawn from the market for clinical use, possibly because of high levels of endotoxins. The endotoxin level in some batches of Percoll is indeed 10–100 times greater than that accepted by the Food and Drug Administration (FDA) (13). Endotoxins have in several studies been shown to reduce the implantation potential of human embryos (14,15). Consequently, new density gradient media were developed containing low levels of endotoxins. In addition, PVP was replaced by silane for coating of the silica particles to make them biologically inert. Comparative studies of Percoll and the new silane-based media have shown comparative performance (16–18).

The aim of the present study was to compare a new medium (SpermFilter) with the established medium on the market (PureSperm) in a non-biased study. The endpoints were purity of the purified spermatozoa (% motile), motility index and recovery rates of progressive motile spermatozoa. In addition, the two media were compared for performance clinically, that is in insemination and *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) programs.

Materials and methods

Density gradient centrifugation and semen processing

100% PureSperm was obtained from Nidacon (Gothenburg, Sweden) and 100% SpermFilter from Cryos International Sperm Bank (Aarhus, Denmark). The media were decanted into sterile, pharmaceutical-grade borosilicate bottles by a third party using sterile techniques. The bottles were identical and were labeled with either "Medium A" or "Medium B." The media appeared identical, so neither the investigator nor the participating clinics knew the identity of the media. The code was broken when the data had been collected, processed and the statistics calculated.

The semen samples were obtained from men undergoing infertility treatment. The semen was allowed to liquefy at 37°C for a maximum of 30 min. The spermatozoa concentration, % motile spermatozoa and motility index (score 0–4) were

analyzed using Makler counting chambers (Sefi Medical Instruments Ltd, Haifa, Israel).

The participating centers were allowed to use the gradient compositions they normally used for purification. Center 1 used 40%/80% (v/v) gradients, center 2 55%/80% (v/v) gradients and center 3 45%/90% (v/v) gradients. All centers used Universal IVF-medium (MediCult, Jyllinge, Denmark) as diluent. The density gradients were performed by layering 1–2 mL of the upper gradient into 15-mL conical Falcon tubes (Falcon 2095, Becton Dickinson). The lower gradients (1–2 mL) underlaid the upper and the semen was loaded on top of the gradients. After centrifugation at 300g for 20 min the seminal plasma, upper gradient layer and most of the lower gradient layer were carefully aspirated. The pellets were gently resuspended in the remaining medium and transferred to new tubes containing 2.5 mL washing medium (Universal IVF Medium). The samples were washed two times at 300g for 10 min before being resuspended in 1 mL Universal IVF medium.

Initially, a total of 225 semen samples were purified using either PureSperm (115 semen samples) or SpermFilter (110 semen samples). Center 1 analyzed a total of 98 semen samples (51 samples using PureSperm and 47 samples using SpermFilter), center 2 analyzed 62 semen samples (30 samples using PureSperm and 32 samples using SpermFilter) and center 3 analyzed 65 semen samples (34 samples using PureSperm and 31 samples using SpermFilter). The two media were tested on alternate dates.

Assisted reproduction

In one of the centers, PureSperm was used clinically for all semen purifications during period I (January to April 2000) while SpermFilter was used during period II only (May to September 2000).

A total of 898 assisted reproductive technology (ART) cycles [245 intrauterine insemination with husband semen (IUI-H), 58 intrauterine insemination with donor semen (IUI-D) and 595 IVF/ICSI cycles] were performed during the two periods. During period I, 133 IUI-H, 33 IUI-D and 266 IVF/ICSI cycles were performed, while in period II, 112 IUI-H, 25 IUI-D and 329 IVF/ICSI cycles were performed. No changes were introduced in the clinical or the laboratory procedures during the course of the study.

Insemination programs

The insemination programs (IUI-H and IUI-D) have been described in detail elsewhere (19,20).

Semen was processed as described above using 40%/80% PureSperm (period I) or SpermFilter (period II) and one intrauterine insemination was performed 38 h following injection of human chorionic gonadotropin (hCG) using an insemination volume of 0.5 mL with a Wallace insemination catheter (H. G. Wallace Ltd, Colchester, Essex, UK). All donor semen was obtained from Cryos International Sperm Bank (Aarhus, Denmark).

IVF/ICSI program

The IVF/ICSI program was performed as described previously (21, 22). Aspirated oocytes were cultured in Universal IVF medium (Medicult) in 5% CO₂ at 95% humidity. Semen was prepared as described above using 40%/80% PureSperm (period I) or SpermFilter (period II) gradients. Oocytes were inseminated 3 h following retrieval with 150 000 or 500 000 spermatozoa per oocyte. In cases of extreme oligozoospermia (<0.5 million purified motile spermatozoa) or previous fertilization failure, ICSI was performed using standard procedures. Oocytes were checked for fertilization 18–20 h after insemination and transferred to fresh IVF medium. At 48 h after retrieval, the developmental stage of the preembryos were checked under an inverted microscope and they were subsequently transferred to M3 medium (Medicult) and cultured for an additional 24 h. At 72 h after oocyte retrieval a maximum of two normally developed preembryos were transferred to the uterine cavity using a Wallace embryo transfer catheter (H. G. Wallace Ltd). Only preembryos with at least six blastomers and less than 20% fragmentation were considered transferable.

Pregnancy test

Blood samples taken 14 days after insemination (IUI) or 17 days after oocyte retrieval (IVF/ICSI) were analyzed by quantitative hCG measurement using the B007-101 AutoDELFI A hCG kit (Wallac Oy). A pregnancy test was considered positive above 50 IU/mL. A transvaginal ultrasonography was performed 4 weeks after a positive pregnancy test and was considered a positive clinical pregnancy if a visible gestational sac with a beating heart was identified.

Endotoxin assay

The endotoxin levels in the two media were measured using the kinetic turbidimetric limulus

amebocyte lysate (LAL) assay following the US FDA guidelines and pharmacopoeia (Scan Dia Laboratory Service, Charlottenlund, Denmark).

Statistics

Power calculation showed that by enrolling 556 IVF/ICSI cycles, the probability is 80% that the study would detect a treatment difference at a two-sided 5% significance level, assuming success rates of 0.2 (20%) and 0.3 (30%) in the two groups. We enrolled 595 IVF/ICSI cycles in the study, so the risk of type 1 and type 2 errors was 0.05 and 0.2, respectively (23).

Data within groups were compared using the nonparametric Wilcoxon rank sum test (paired data). Data between groups were analyzed using the Mann–Whitney *U*-test (unpaired data). The χ^2 -test was used for categorical data. A *p*-value <0.05 was considered statistically significant.

Results

The characteristics of the semen samples used for evaluation of the two density media are summarized in Table I. PureSperm was used for purification of 115 semen samples while SpermFilter was used for 110 semen samples. The semen volume, concentration of spermatozoa, % motile spermatozoa and motility score in the two groups were not statistically different. The results after purification are also shown in Table I. No statistical difference between the two media could be demonstrated. There was a significant increase in percent motile spermatozoa after purification with both PureSperm and SpermFilter compared with that in the raw semen, before purification (*p* < 0.01). The motility index and the percent motile spermatozoa after purification were the same with the two media. The recovery rates (37.5% and 37.4%), calculated as the ratio between the total number of motile spermatozoa present after purification and the total number of motile spermatozoa before purification, were also similar.

In center 1, the media were also tested for performance in clinical assisted reproduction. PureSperm was used during January to April 2000 for purifying all semen samples used in connection with IUI-H, IUI-D and in IVF/ICSI cycles. During period II (May to September 2000), PureSperm was replaced with SpermFilter.

As shown in Tables II–III, there were no differences in ages and infertility diagnoses of the patients in the study.

The IUI-H results are shown in Table II. A total of 245 inseminations were performed during

Table I. Data before and after purification in the two groups. Data are means. All data, $p > 0.05$

	PureSperm	SpermFilter
Number of semen samples	115	110
Data before purification		
Semen volume (mL) (95% CI)	3.4 (3.2–3.7)	3.5 (3.2–3.9)
Spermatozoa concentration (million/mL) (95% CI)	51.7 (42.2–61.1)	59.1 (48.9–69.2)
% motile spermatozoa (%) (95% CI)	50.5 (46.6–54.8)	54.5 (51.6–57.7)
Motility (0–4) (95% CI)	2.7 (2.3–3.2)	2.8 (2.4–3.3)
Total motile spermatozoa (million) (95% CI)	88.7 (70.4–107.1)	112.8 (88.1–137.4)
Data after purification		
% motile spermatozoa (95% CI)	82.6 (78.9–86.3)	87.9 (85.5–90.4)
Motility (0–4) (95% CI)	3.2 (3.1–3.2)	3.3 (3.2–3.4)
Total motile spermatozoa (million) (95% CI)	33.3 (22.7–48.7)	42.2 (21.8–52.9)
Recovery of motile spermatozoa (%) (95% CI)	37.5 (32.4–42.5)	37.4 (31.8–43.0)

the two periods with 133 cycles in period I and 112 cycles in period II. The mean number of spermatozoa available for insemination (31.0 and 26.1 million, respectively) and the number of cycles with less than 5 million spermatozoa available for insemination during period I (16.5% of the cycles) and period II (26.8% of the cycles) were also not statistically different. Comparative positive pregnancy rates (21.1% and 24.3%, respectively), clinical pregnancy rates (20.3% and 23.2%, respectively) and baby take-home rates (18.0%, 22.3%) per insemination were obtained using the two density media (no statistical differences). There were no malformations detected.

The same tendency was obtained in the IUI-D program (data not shown) encompassing 33 inseminations using PureSperm and 25 insemination cycles using SpermFilter, resulting in positive pregnancy rates of 21.2% and 24.0%, respectively (NS). Again, no malformations were reported.

During period I, 266 IVF/ICSI cycles were performed using PureSperm for all semen processing, while during period II, 329 IVF/ICSI cycles were performed using SpermFilter only (Table III). Of the 266 cycles performed during period I, ICSI was performed in 67 cycles (26.7%), while ICSI was used in 92 cycles (29%) during period II (NS). The overall fertilization rates (IVF and ICSI) were 70.6% and 69.8% using the two media (NS). The number of transferable embryos, defined as at least six blastomers and less than 20% fragmentation on day 3 following retrieval, were 27.2% and 26.7% per fertilized oocyte, respectively (NS). The numbers of transferred embryos in the two groups were also the same. The positive pregnancy rates per transfer were 32.9% and 33.8% (NS), the implantation rates 19.8 and 21.0 (NS), the clinical pregnancy rate per cycle 23.6% and 24.6% (NS) and the baby take-home rates were 21.4% and 20.4% (NS), respectively. Again, no malformations were reported in the children born.

Table II. Results obtained on insemination with husband semen (IUI-H) using PureSperm (period I) or SpermFilter (period II). All data, $p > 0.05$

	PureSperm	SpermFilter
Mean age of patients (years) (range)	30.6 (21–40)	30.9 (19–40)
Infertility diagnosis (<i>n</i> , %)		
Idiopathic	58 (43.6)	39 (34.8)
Male factor	35 (26.3)	35 (31.3)
Polycystic ovarian syndrome (PCO)	12 (9.0)	10 (8.9)
Other (tubal factor, endometriosis, etc.)	28 (21.1)	28 (25.0)
No. of inseminations	133	112
Mean no. of spermatozoa per insemination (million)	31.0	26.1
No. of cycles with < 5 million motile spermatozoa	22	30
Positive pregnancies (<i>n</i> , %)	28 (21.1)	27 (24.1)
Clinical pregnancies (<i>n</i> , %)	27 (20.3)	26 (23.3)
Abortions	2	1
Births	25	25
Singletons	20	19
Twins	5	5
Triplets	0	1
Neonatal death	1 (singleton)	1 (twin pregnancy)
Baby take-home rate (living births/insemination) (%)	18.0	22.3

Table III. Results obtained in the IVF/ICSI program with PureSperm (period I) and SpermFilter (period II). All data, $p > 0.05$

	PureSperm	SpermFilter
Mean age of patients (years) (range)	31.4 (21–40)	31.5 (21–41)
Infertility factor (<i>n</i> , %)		
Tubal	99 (37.2)	103 (31.3)
Male	99 (37.2)	131 (39.8)
Idiopathic	49 (18.4)	64 (19.5)
PCO	11 (4.1)	25 (7.6)
Other cause	8 (3.0)	6 (1.8)
Started treatments	266	329
Aspirations	251	318
Transfers	228	278
Mean no. of aspirated oocytes	11.9	12.3
Fertilization rate (%)	70.6	69.8
Transferable embryos/aspirated oocytes (%)	27.2	26.7
Embryos/transfer	1.89	1.89
Pregnancies	75	94
Positive pregnancies/transfer (%)	32.9	33.8
Gestational sacs	82	110
Implantation rate (%)	19.8	21.0
Clinical pregnancies	62	81
Clinical pregnancy rate/cycle (%)	23.3	24.6
Abortions	5	13
Births	57	68
Singletons	43	46
Twins	14	21
Triplets	0	1
Neonatal death	1 (twin pregnancy)	1 (singleton)
Malformations	0	0
Baby take home rate (living births/started cycle) (%)	21.4	20.4

Discussion

The present study investigating the performance of two density gradient centrifugation media for purifying spermatozoa for assisted reproduction shows similar outcome for all parameters investigated.

The design of the first part of the study was blinded. The clinics received identical anonymous bottles, the only difference being that the two bottles were labeled "Medium A" or "Medium B." The media appeared identical both in color, chemical behavior and density. Parameters, measured by many fertility centres (i.e. pH and osmolality), were for both media in the normal range. The endotoxin levels in both media were below 1 EU/mL. Thus the investigating clinics were not able to measure any differences between the two media.

Evaluating a total of 225 semen samples in this multicenter study showed no differences in any of the parameters investigated, that is percent motile spermatozoa, motility index, total number of spermatozoa purified and recovery rate of motile spermatozoa

The three clinics used three different densities for gradient centrifugation: 40%/80% (v/v), 55%/80% (v/v) and 45%/90% (v/v). When comparing the data from the individual clinics no

significant differences were demonstrated, except that the clinic using 40%/80% gradients had slightly better recovery rates of motile spermatozoa (data not shown). This may indicate that the 40%/80% gradient is better than the other gradient combinations, although the difference could be due to the handling. To our knowledge, no systematic study has been published comparing the densities used in this study. However, the same tendency was observed comparing 40.5%/81% with 47.5%/95% Percoll gradients. The former gave higher yields compared with the latter (35.4% vs. 30.0%) (24, 25). This was also observed comparing 40%/80% and 45%/90% gradients, and it was concluded that it is especially important to use the 40%/80% combination in connection with oligo- and/or astenozoospermia because of the significantly better recoveries of motile spermatozoa (26).

The overall mean recovery rate of motile spermatozoa after purification was 37% independent of the medium used. Others have found similar recovery rates (1, 16, 27), but also lower recovery rates of 20% (28) and 10.5% (18) have been reported.

The fact that differences in the purification data between the centers were only marginal indicates that semen processing, using slightly

different density combinations, has little effect on the outcome. This indicates that semen purification by density centrifugation using silane-coupled silica-based media is a robust method and thus very suitable for semen purification despite the differences among laboratories worldwide.

When comparing the two media in a clinical setup, the outcome was similar, irrespectively of the treatment provided. Inseminations with husband semen were performed in a total of 245 cycles and insemination with donor semen in 54 cycles. In both cases, the results showed no significant differences in baby take-home rates.

When the two media were compared in a total of 595 IVF/ICSI cycles the same tendency was observed. Power calculation showed that by enrolling more than 556 IVF/ICSI cycles the study had a power of 80% to detect a difference of 10%. Using PureSperm for all semen purifications in combination with IVF/ICSI in 266 cycles gave a fertilization rate per aspirated oocytes of 70.6%, an implantation rate of 19.8% and a baby take-home rate of 21.4% per cycle. Using the new medium, SpermFilter, a fertilization rate of 69.8%, an implantation rate of 21.0% and a baby take-home rate of 20.4% were obtained. These results are very similar and not statistically different. The number of transferable embryos, defined as at least six blastomers and less than 20% fragmentation on day 3, per aspirated oocyte was 27% with both media. This, in connection with the same implantation rates obtained (19.8% vs. 21.0%), indicates that PureSperm and SpermFilter behave in an identical manner in IVF/ICSI programs and are possibly nontoxic to gametes and embryo development, which is also substantiated by the fact that no malformations were observed during the course of the study.

Alternative density gradient media for semen purification have been developed based on iodixanol (Optiprep, Axis-Shield PoC AS, Oslo, Norway) (29,30); however, the silica-based media seem to be superior in terms of percent motile spermatozoa, spermatozoa velocities, recovery rates and percent morphological normal spermatozoa purified (16).

In clinical assisted reproduction it is essential to use very pure products to avoid any harmful effects on the gametes, embryos and patients. In addition, it is important to ensure that it is efficient and suitable for its intended use and gives results at least as good as established products on the market. Both requirements are fulfilled with the new medium, SpermFilter, described here.

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