Seaforia™ Sperm Separation System

Scientific Data

Seaforia™

LotusBIO®

Scientific Data

At the end of the *Seaforia*™ separation procedure, the medium that initialy contained no sperm cells, becomes sperm-cells enriched. The isolated sperm cells population is characterized by progressive motility and a high percentage of normal morphology (Figure and Table 1).

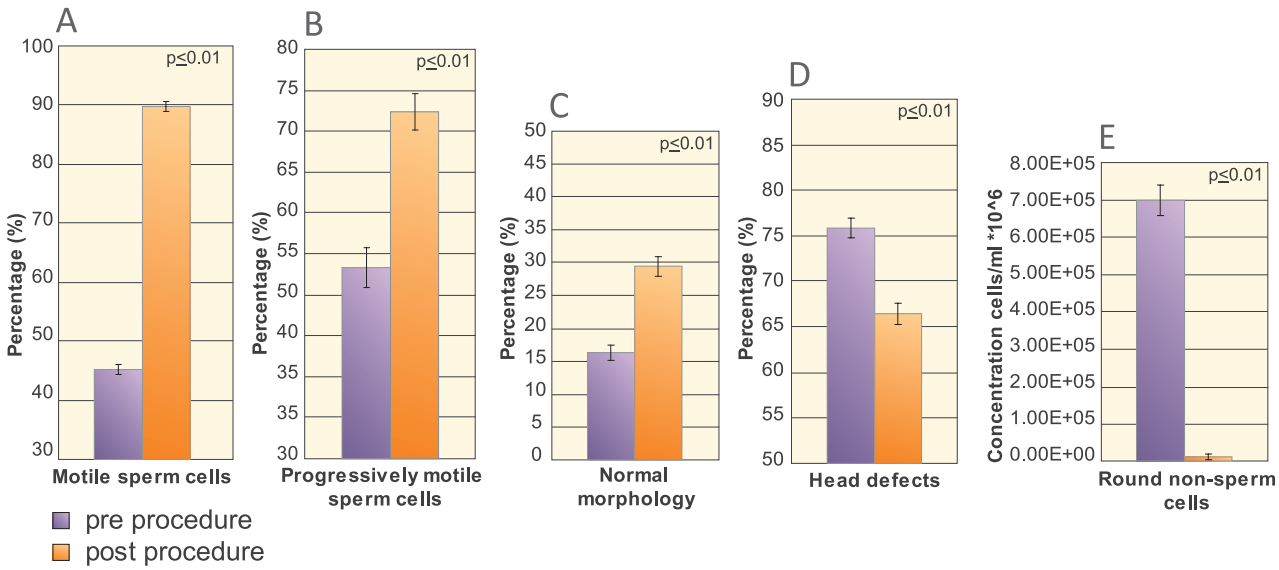


Figure 1 (A-E): Semen parameters pre- and post- *Seaforia*™ procedure. Sperm cells preparation procedure was conducted using separators with different semen volumes. The semen was evaluated according to standard assessment (WHO guidelines), pre and post separation procedures.

Using *Seaforia*™ Sperm Separation System in an expanded test (n=75) showed a significant difference in the pre- and post-procedure fractions for the following parameters: Percentage of motile sperm cells (about 90% of the isolated sperm cells were motile, A); percentage of progressively motile sperm cells (about 73% of the isolated motile sperm cells had progressive motility, B); percentage of morphologically normal sperm cells (about 29% of the isolated sperm cells had normal morphology, C); combined with a decrease in head defects (D); and round non-sperm cells concentration (decrease in round non-sperm cells in the post-separation fraction, E). Results are mean ± s.e.

There is a significant improvement of the morphologically normal spermatozoa post-procedure in comparison to the pre-procedure fraction. Concomitantly, there is a significant decrease in the percentage of head defects post-procedure relative to the pre-procedure population.

Table 1: T-test comparison of basic sperm cells parameters pre- and post-*Seaforia*™ Sperm Separation fractions using separators with different volumes of inserted semen sample.

Sperm parameter	Pre M ± SE	Post M ± SE	p≤
Percent of motile spermatozoa (%), n=75	45.26±1.08	89.81±0.9	0.01
Percent of progressively motile spermatozoa (%), n=75	53.77±2.27	72.67±1.9	0.01
Morphologically normal spermatozoa (%), n=64	16.31±1.12	29.45±1.6	0.01
Percent of head defects (%), n=61	75.80±1.16	66.43±1.3	0.01
Round non-sperm cells ([cell/ml]*10^6), n=75	0.7±0.05	0.01±0.004	0.01

An elite quality population of sperm cells isolated by the *Seaforia*™ System demonstrates higher efficacy than "Total motile sperm cells threshold"

From a literature review it is clear that there is a wide range regarding the recommended threshold of total motile sperm cells (TMSC) as an outcome of sperm preparation procedure for IUI and IVF. TMSC ranges from $0.8x10^6$ to $5x10^6$ [Van Voorhis BJ (2010). F, V & V IN OBGYN, MONOGRAPH: 29-31].
An internal survey among professional people within the fertility arena supports a practical threshold of $5x10^6$ TMSC, hence it was chosen as a minimal threshold parameter for the *Seaforia*™ Sperm Separation procedure efficacy.

Figure 2 presents the suggested threshold of $5x10^6$ total motile sperm cells as an outcome of sperm preparation of original semen sample which was chosen as a minimal threshold parameter for the *Seaforia*™ Sperm Separation System. 23 semen samples with motile sperm cells concentrations ranging from $8.13\text{-}22.2x10^6$ (cells/ml) were randomly selected.

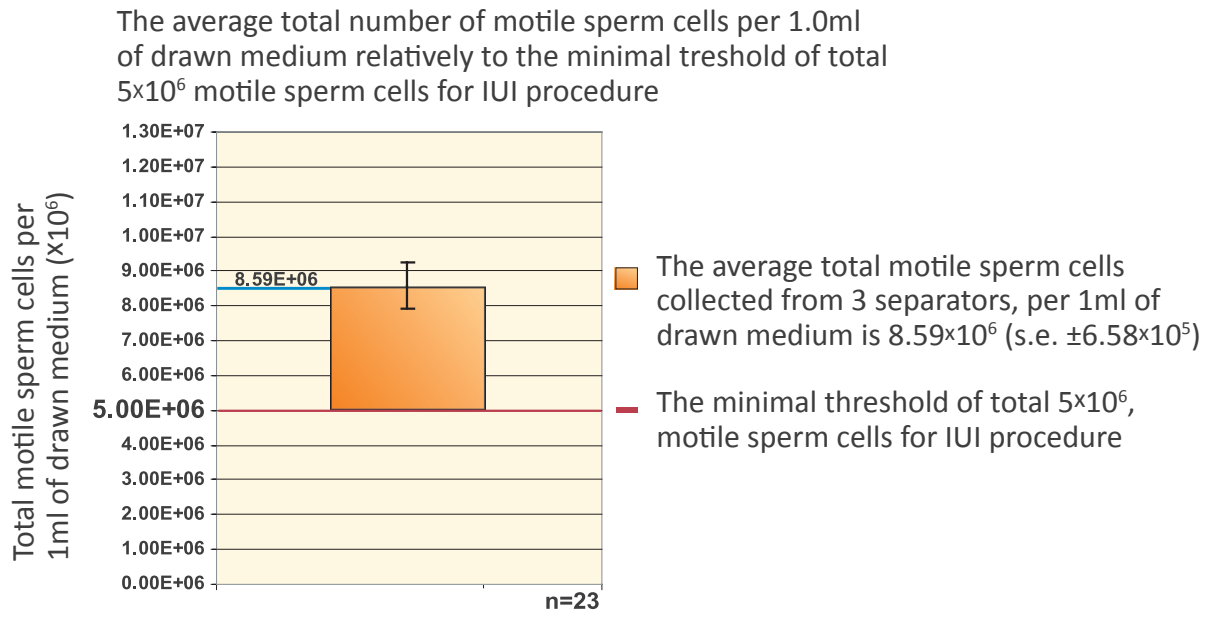


Figure 2: The average total no. of motile sperm cells in drawn medium (calculated per 1ml) post *Seaforia*™ Sperm Separation procedure (using 3 separators of 1ml inserted semen sample) at the 23 repetitions, relative to a minimal threshold of $5x10^6$ motile sperm cells for an IUI procedure.

According to the threshold of $5x10^6$ total motile sperm cells as an outcome of sperm preparation procedure of an original semen sample, the average total motile sperm cells collected from 3 separators, per 1ml of drawn medium is $8.59x10^6$ (s.e $\pm 6.58x10^5$, n=23), having average progressive motility of 72.67% (s.e $\pm 3.43x10^6$, n=23, Figure 2).

These features demonstrate that the novel *Seaforia*™ System is an excellent vehicle for selectively isolating, in a natural way, the best sperm cells population for fertilizing the ovum using intra-uterine insemination or in vitro fertilization.

A comparison between the *Seaforia*™ Sperm Separation procedure and two gold standard sperm preparation procedures (discontinuous density gradient and direct swim-up procedures)

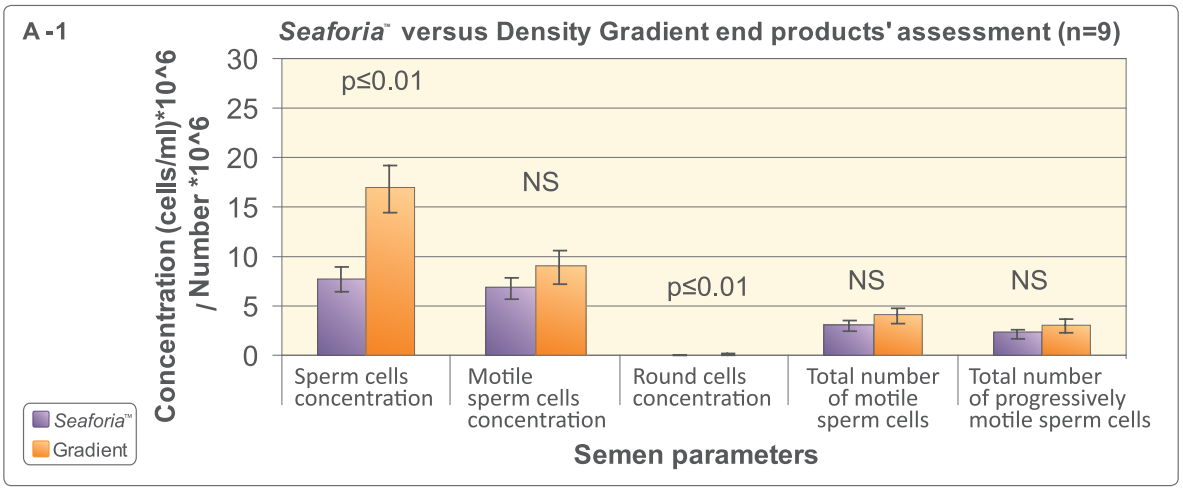
Two of the most common sperm preparation procedures are:

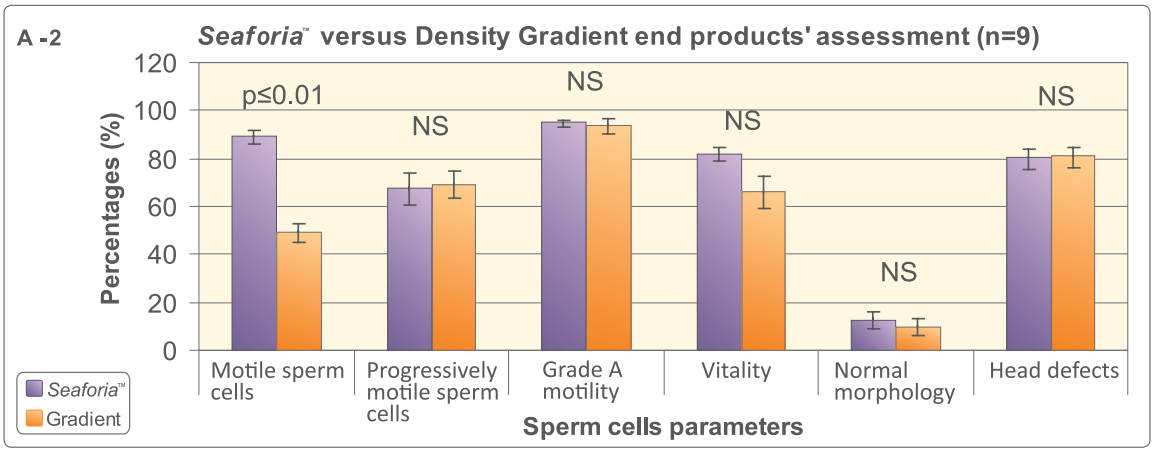
1. Direct swim-up, in which spermatozoa are selected by their ability to swim out of the seminal plasma, by layering culture medium over the liquefied semen. Motile spermatozoa then swim into the culture medium

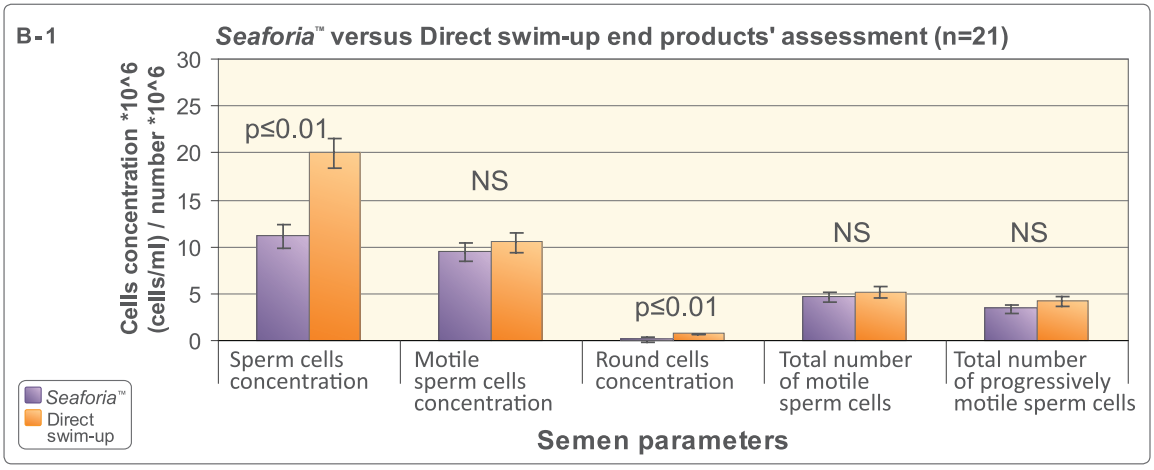
2. Discontinuous density gradient, in which cells are separated by their density and sperm cells are actively swimming through the gradient material by layering semen sample over density gradient (WHO, 2010[1]).

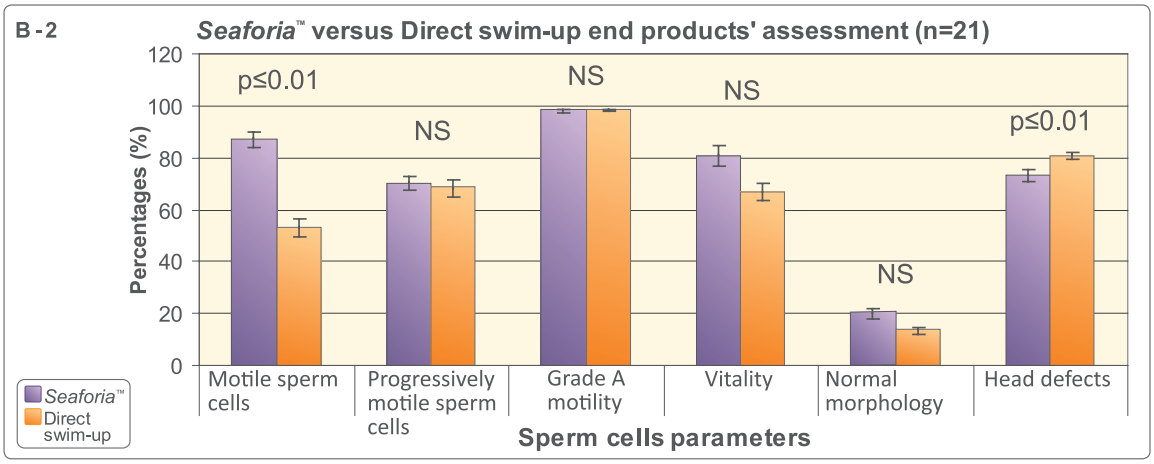
The two gold standard procedures require laboratory equipment such as a centrifuge and an incubator, and should be conducted by a qualified technician. It is recommended in the WHO (2010)[2] to minimize the use of centrifugation as much as possible.

Since the *Seaforia*™ procedure does not require a centrifugation, a comparison to the two gold standard procedures' efficacies was planned:
Each semen sample was evaluated by routine laboratory methods and then was used to conduct the preparation procedures. The end products of the preparation procedures were assessed post procedures and post 24 hours of incubation within a closed vial in 37°C. The results (presented in graph 1 and table 1) were analyzed using a student t-test.









Graph 1: A comparison of sperm cells preparation using *Seaforia*™ Sperm Separation System versus discontinuous density gradient (n=9, A-1&2) and versus direct swim-up (n=21 from 2 different laboratory tests, B-1&2). The same original semen sample was divided into two or three and subjected to sperm cells separation using *Seaforia*™ Sperm Separation procedure, Direct Swim-up and Discontinuous density gradient. The semen sample was evaluated according to standard assessment (WHO, 1999, 2010) pre-and post- separation procedures. The final pellets of the discontinuous density gradient and of the direct swim up techniques were re-suspended with the same volume as of the *Seaforia*™ Sperm Separation procedure's end product volume.

Seaforia™ versus Density Gradient & Direct swim-up end products' assesment - post 24 hours

Table 1:
A comparison between *Seaforia*™ procedure end product's assessment to the discontinuous density gradient and swim-up procedures end products' assessments – all post 24h incubation within a closed vial in 37°C

The parameter		Density gradient procedure post 24h (n=9)			Direct swim-up post 24h (n=5)		
		Seaforia™ procedure average (mean±s.e)	Average (mean±s.e)	p≤ (vs *Seaforia*™ procedure)	*Seaforia*™ procedure average (mean±s.e)	Average (mean±s.e)	p≤ (vs *Seaforia*™ procedure)
Sperm cells concentration $\times 10^6$ (cells/ml)		6.8±1.2	20.7±3.7	0.01	6.4±1.7	24.4±5.1	NS
Motile sperm cells concentration $\times 10^6$ (cells/ml)		0.6±0.3	4.4±2.1	NS	1.1±0.5	0.8±0.8	NS
Motile sperm cells percentage (%)		13.0±6.7	18.0±6.2	NS	23.5±10.1	2.6±2.6	NS
Progressively motile sperm cells percentage (%)		22.8±11.7	51.0±13.4	NS	41.0±17.8	17±17.3	NS
Grade A motility percentage (%)		22.9±13.2	52.0±14.6	NS	40.0±21.3	14±14.2	NS
Round cells concentration $\times 10^6$ (cells/ml)		0.03±0.03	0.1±0.04	NS	0.05±0.05	0.7±0.02	NS
Total motile sperm cells $\times 10^6$		0.2±0.1	2.1±0.9	NS	0.4±0.2	0.3±0.3	NS
Total progressively motile sperm cells $\times 10^6$		0.2±0.1	1.8±0.9	NS	0.3±0.2	0.3±0.3	NS
Volume (µl)		453.3±15.4	453±15.4	-	434.0±14.9	434.0±14.9	-
Sperm cells vitality percentage (%)		35.9±10.0	45.0±7.1	NS	52.4±12.4	16.0±10.0	0.01
Sperm cells morphology (%)	Normal	14.1±3.4	13.0±3.7	NS	12.8±4.8	14.8±6.4	NS
	Head defects	77.8±3.8	82.0±3.2	NS	80.0±4.7	76.0±6.7	NS
	Mid piece defects	7.0±1.6	7.0±1.5	NS	6.4±2.0	7.2±2.5	NS
	Tail defect	19.0±6.3	28.0±4.5	NS	24.6±10.8	28.4±2.0	NS
	Cytoplasmic droplet	0±0	0±0	NS	0±0	0.4±0.4	NS

Summary

1. Comparing *Seaforia*™ procedure versus discontinuous density gradient procedure:
    a. *Seaforia*™ procedure is as good as discontinuous density gradient procedure for sperm preparation concerning parameters as:
        I. Motile sperm cells concentration
        II. Percentage of grade A motile sperm cells
        III. Percentage of progressively motile sperm cells
        IV. Percentage of normal morphology sperm cells
        V. Percentage of sperm cells with head defects.
        VI. Total number of motile sperm cells.
        VII. Total number of progressively motile sperm cells.
    b. *Seaforia*™ procedure displays an advantage upon discontinuous density gradient procedure concerning parameters as:
        I. Percentage of motile sperm cells
        II. Round cells concentration
    c. In the discontinuous density gradient procedure the parameter of sperm cells concentration is higher than *Seaforia*™ procedure. This parameter includes immotile and dead sperm cells and therefore it is a disadvantage.
    d. Post 24 h *Seaforia*™ procedure's end product is as good as discontinuous density gradient procedure's end product for sperm preparation concerning all the parameters detailed in paragraphs a and b.
2. By comparing *Seaforia*™ procedure versus swim-up procedure:
    a. *Seaforia*™ procedure is as good as swim-up procedure for sperm preparation concerning parameters as:
        I. Motile sperm cells concentration
        II. Percentage of grade A motile sperm cells
        III. Percentage of progressively motile sperm cells
        IV. Percentage of normal morphology sperm cells
        V. Total number of motile sperm cells.
        VI. Total number of progressively motile sperm cells.
    b. *Seaforia*™ procedure shows an advantage upon swim-up procedure concerning parameters as:
        I. Percentage of motile sperm cells
        II. Round cells concentration
        III. Percentage of sperm cells with head defects.
    c. In the swim-up procedure the parameter of sperm cells concentration is higher than *Seaforia*™ procedure. This parameter includes immotile and dead sperm cells and therefore it is a disadvantage.
    d. Post 24 h *Seaforia*™ procedure is as good as swim-up procedure for sperm preparation concerning all the parameters detailed in paragraphs a, b and c.

Conclusion

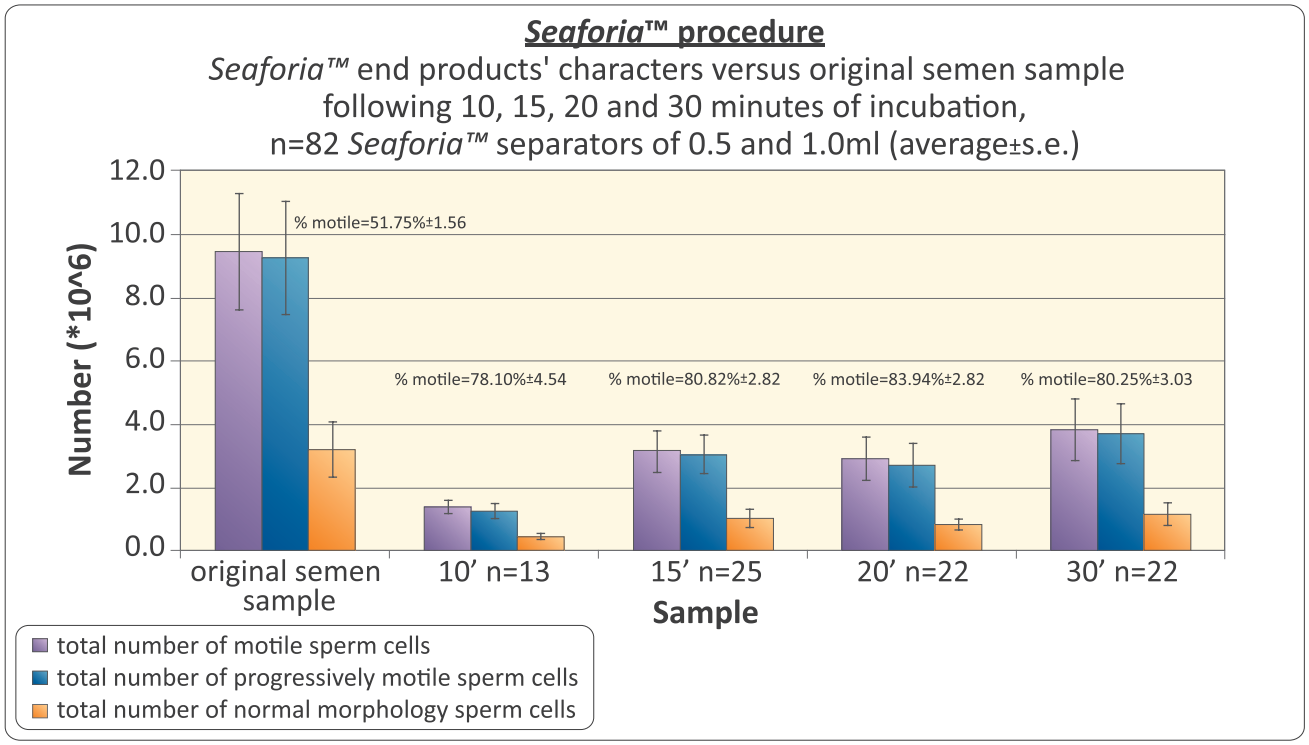
The *Seaforia*™ Sperm Separation System for sperm preparation (in the natural way without centrifugation) is at least as good as the two most common sperm preparation procedures (swim up and discontinuous density gradient) by end products' comparison.
The *Seaforia*™ procedure requires no additional lab equipment or mechanical handling such as centrifugation. Since the WHO (2010) recommends minimizing the use of centrifugation as much as possible, the *Seaforia*™ procedure demonstrate a major advantage.

Seaforia™ end products' characters following 10, 15, 20 & 30 minutes of incubation, n=82 *Seaforia™* separators of 0.5 & 1.0ml

VERSION ③

Twenty semen samples characterized by sperm cells concentration range of 5.63-58.4×10^6 cells/ml, motile sperm cells concentration range of 2.81-36.6×10^6 cells/ml and normal morphology percentage range of 6-32% (in average 28.8×10^6±4.08×10^6, 15.30×10^6±2.38×10^6 and 16.95%±1.60%, respectively) were utilized for *Seaforia™* procedure by Lotus Bio's staff using 82 separators of 0.5 and 1.0ml.

The end products were assessed following 10, 15, 20 and 30 minutes of incubation. Their characters (such as percentage of motile sperm cells, progressively motile sperm cells and normal morphology, and round cells concentration) were improved relatively to the original semen sample (data not shown). On average the total number of motile and progressively motile sperm cells and of total number of normal morphology sperm cells after 15 minutes (and 20 minutes) were similar to the results after 30 minutes of incubation (3.16×10^6±0.66×10^6, 3.03×10^6±0.66×10^6 and 1.02×10^6±0.33×10^6, respectively. See table 1 and graph 1).



Graph 1: The assessed characters of the *Seaforia™* end products following varied incubation periods

Table 1: *Seaforia™* end products' characters using 0.5 & 1ml separators following 10, 15, 20 & 30 min. of incubation (mean ± s.e.)

The parameter	Original semen	*Seaforia™* end products' characters utilizing 0.5 and 1.0ml separators			
		10 min. incubation	15 min.incubation	20 min.incubation	30 min.incubation
Total no. of motile sperm cells (cells, ×10^6)	9.49±1.85	1.38±0.25	3.16±0.66	2.91±0.70	3.85±0.97
Total no. of progressively motile sperm cells (cells, ×10^6)	9.27±1.82	1.26±0.24	3.03±0.66	2.72±0.71	3.70±0.97
Total no. of normal morphology sperm cells (cells, ×10^6)	3.22±0.88	0.45±0.12	1.02±0.33	0.84±0.21	1.18±0.38
Motile sperm cells percentage (%)	51.75±1.56	78.10±4.36	80.82±3.19	83.94±2.82	80.25±3.03

* Since for IVF aplications sperm cells having good motility and morphology are needed, therefore the *Seaforia™* procedure`s end product following 15 min. of incubation is recomended for IVF purposes.

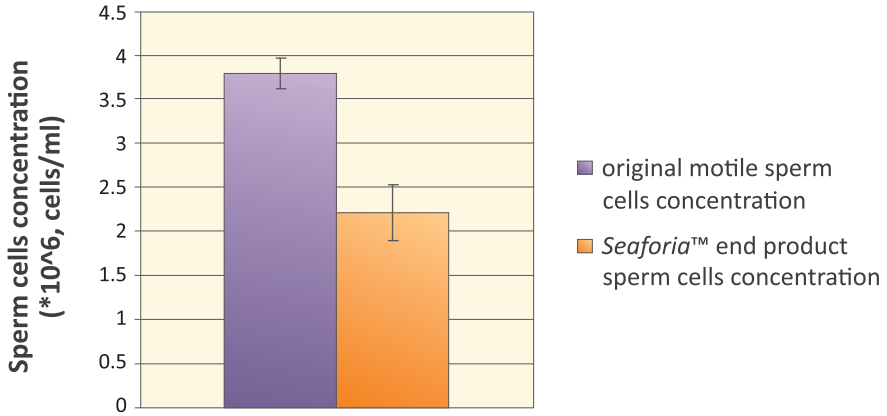
VERSION ②

The decline of the semen quality over the years is expressed also by the severity of asthenozoospermia; a study of 3729 men demonstrated that there is a significant decrease in the percentage of sperm motility in the 2000s compared with the 1980s [Mukhopadhyay D et al (2010). Ferti Steril 93: 2247-2254].

Eighteen semen samples characterized by motile sperm cells concentration lower than 5×106 cells/ml (range of 0.31×10^6-4.69×10^6 motile cells/ml, in average 3.80×10^6±0.19×10^6) were utilized for *Seaforia*™ procedure by Lotus Bio's staff using 30 separators of 0.5 and 1.0ml.

The end products were assessed following 30 minutes of incubation. Their characters (such as percentage of motile sperm cells, progressively motile sperm cells and normal morphology, concentration of round cells) were improved relatively to the original semen sample (data not shown). On average the end product's sperm concentration was 2.21×10^6±0.32×10^6, see table 1 and graph 1).

Seaforia™ procedure efficacy for semen samples characterized by low concentration of motile sperm cells (<5x10^6, n=30, ± s.e.)



Graph 1: The *Seaforia*™ end products (utilizing separators of 0.5 and 1.0 ml) sperm cells concentration versus original semen sample motile sperm cells concentration.

Table 1: *Seaforia*™ end products' sperm cells concentration utilizing 0.5 ml and 1.0 ml (mean ± s. e.) versus original semen sample motile sperm cells concentration (n=30)

Original semen sample motile sperm cells concentration (×10^6, cells/ml)	*Seaforia*™ end products' sperm cells concentration (×10^6, cells/ml)
3.80×10^6±0.19×10^6	2.21×10^6±0.32×10^6

The *Seaforia*™ procedure is effective utilizing semen samples with motile sperm cells concentration <5×10^6 while using separators of 0.5 and 1.0ml and 30 min incubation.

FSA - 2018

The effects of semen processing using three methods on sperm ROS and apoptosis

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Aim: This study aims to evaluate the effects of semen processing on sperm reactive oxygen species (ROS) production and apoptosis using three different methods: density gradient centrifugation (DGC), swim-up and Seaforia™.

Method: Following sample collection by masturbation and routine semen analysis, excess normospermic semen was pooled (n = 17). Each sample was aliquoted for processing using DGC, swim-up and Seaforia™. ROS(+) and apoptotic sperm populations were measured on neat and processed specimens of each sample using dihydroethidium (DHE) and Annexin V assays respectively. Statistically significant differences among processing methods were determined using one-way repeated measure ANOVA.

Results: The results revealed that DGC (2.36% □ 1.06 and 5.42% □ 4.61), Seaforia™ (2.68% □ 1.47 and 3.79% □ 2.51) and swim-up (2.87% □ 1.45 and 4.93% □ 3.51) effectively reduced both early and late apoptotic sperm from the neat samples (15.4% □ 1.39, 11.26% □ 5.09, respectively). No significant difference was observed between three methods ($p > 0.05$). In comparison to the neat sample (17.30% □ 7.00), a significant reduction in ROS(+) population was observed after processing using Seaforia™ (8.22% □ 5.57) and swim-up (9.51% □ 5.18), but not DGC (18.71% □ 13.68). There was no significant difference between neat and DGC samples, and between Seaforia™ and swim-up ($p > 0.05$). It was also observed that Seaforia™ produced samples with higher sperm concentration compared to swim-up.

Conclusion: Our findings suggest that although all three sperm processing methods are equally effective in reducing both early and late apoptotic sperm, swim-up and Seaforia™ are superior in eliminating ROS(+) population from the neat samples.

SUITABILITY OF THE SEAFORIA™ SPERM SEPARATION SYSTEM FOR USE IN ART

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Objective: To evaluate the suitability of routine use of the SeaforiaÊ sperm separation system in assisted reproductive technologies (ART).

Design: Prospective evaluation using random semen specimens.

Materials and Methods: From 38 semen samples an aliquot was snap frozen for sperm DNA fragmentation (SCSA) testing and a second aliquot was prepared using the SeaforiaÊ system following the manufacturerôs protocol. Both the initial specimen and the final sperm preparation were assessed for sperm concentration, motile sperm recovery rates, and sperm DNA fragmentation. General comments regarding the ease and suitability of the protocol from laboratory staff were collected.

Results: There was a significant difference in sperm concentration between raw semen (76.8 million / ml, range 25 ï 150 million / ml) and Seaforia prepared samples (15.6 million / ml (range 0.5 ï 75.0 million / ml), $P < 0.05$). There was also a significant difference in mean SCSA DNA Fragmentation Index between raw semen (9.92%, range 3.35% - 28.83%) and following SeaforiaÊ preparation (5.25%, range 0.76% - 22.95%), $p = 0.03$.

The mean recovery of motile sperm following preparation was 45.0% (range 6.3% - 80.0%). 34 of the 38 final sperm preparations contained 100% motile sperm. There was a significant improvement in the mean total motility seen in the SeaforiaÊ preparation (96.5%) compared to the raw semen samples (75.4%), $p < 0.05$.

Conclusions: Preparation of semen samples using the SeaforiaÊ system can yield sperm samples that have a significant improvement in sperm DNA fragmentation and sperm motility. Hence the Seaforia system is suitable for use in all forms of assisted reproductive technologies. The system is highly user friendly which combined with the fact that centrifugation is not required means that sperm samples for IUI could be prepared in a minimally equipped laboratory.

Support: Seaforia sperm separation separators used in this study were provided by Lotus Bio (Nymphaea) Ltd through the Australian distributor Gytech Ltd.

Comparison of the Seaforia™ Sperm Preparation system with Density Gradient Sperm Preparation

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Aim: To compare the SeaforiaÊ sperm preparation system with a density gradient centrifugation method that is routinely used in the preparation of semen samples for IVF.

Method: Semen samples with varying sperm concentrations and motility that had been deemed suitable for IVF, were divided into aliquots. One aliquot was prepared using the laboratoryÕs standard density gradient centrifugation protocol. A second aliquot was prepared with the SeaforiaÊ sperm preparation system using the protocol supplied by the manufacturer.

Final sperm preparations were assessed for percentage of motile sperm recovered, sperm concentration and total sperm motility.

General comments regarding the ease and suitability of the protocol to laboratory staff were collected.

Results: Two semen samples were excluded from the study as the SeaforiaÊ system was unable to be utilised due to the viscosity.

Of the 38 samples assessed using density gradient centrifugation and SeaforiaÊ system, there was no significant difference in the motile sperm recovery rate (Density Gradient 43.89% vs SeaforiaÊ 46.74%). Similarly there was no significant difference between the total sperm motility in the final two preparations (>90% motility). Nor was there a significant difference in the mean concentration of the final sperm preparation (Density Gradient 14.6 Million/mL vs SeaforiaÊ 15.6 Million/mL).

Conclusion: The SeaforiaÊ sperm preparation system yields equivalent quality sperm to density gradient centrifugation. Sperm that is highly viscous must be pretreated prior to processing using the SeaforiaÊ sperm preparation. SeaforiaÊ is easy to use and is a valid alternative to density gradient centrifugation when used for the preparation of samples for IVF/IUI.

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CAT10260 Rev 1.1 15/10/18

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