

Introducing the Felix™ Sperm Separation System

by Robert John Aitken and Farnaz Shapouri

In 2007, the world witnessed the first recorded pregnancy using a unique sperm isolation technique that selected sperm on the basis of their size and charge rather than the traditional criteria of sperm density or motility (*Ainsworth et al., 2007*). The couple in question had experienced multiple cycles of ICSI characterized by high rates of fertilization but poor embryo development. The male was oligozoospermic ($3.2 \times 10^6/\text{ml}$), severely asthenozoospermic (18% motility) and had extremely high levels of sperm DNA damage (41% DNA fragmentation index according to SCSA [Sperm Chromatin Stability Assay]). Following electrophoretic separation, the DNA damage was reduced to 15%, the motility improved to 24% and both fertilization and subsequent embryo development were successfully achieved. Subsequent embryo transfer resulted in the birth of a normal healthy girl.

Following this initial success, a more extensive clinical trial was performed at Westmead Hospital in Sydney on 28 couples during which oocytes were inseminated with sperm that had been isolated by electrophoresis or density gradient centrifugation (DGC). Fertilization and embryo development rates were monitored and the highest quality embryos were transferred, regardless of the mode of sperm isolation. The results of this analysis demonstrated that both methods of sperm isolation were associated with equivalent levels of fertilization, cleavage and development of high-quality embryos (*Fleming et al, 2008*). In this preliminary analysis, pregnancy rates were actually higher (33%) with the electrophoretically isolated sperm compared with their DGC isolated counterparts. We concluded from this initial trial that the electrophoretic method of sperm isolation was at least as good as the traditional method of DGC sperm isolation whilst taking a fraction of the time.

This method of electrophoretic sperm isolation was subsequently patented but multiple attempts to generate a commercial product were unsuccessful until Memphasys Ltd took over the development of this system in 2015. With their engineering partners, Hydrix, Memphasys have turned this general concept into a functional, practical instrument, the Felix™ System, that was successful in winning two Good Design Awards in 2020.



The Felix™ System, with cartridge being inserted

The Felix™ System separates sperm from raw semen by a proprietary process combining electrophoresis and size exclusion membranes that allow high quality sperm into the harvest chamber while excluding cellular contaminants such as leucocytes and precursor germ cells.

The Felix™ System is now being evaluated in a number of independent reproductive healthcare clinics across the globe, with a view to generating *in vitro* data on the performance of the Felix device relative to the conventional methods of sperm isolation, DGC and Swim-Up (S-U)

In the first series of *in vitro* tests, sperm isolation with the Felix™ System and DGC was compared in 5 independent clinics (Coimbatore (India) Monash IVF (Australia), New York (USA), Gothenburg (Sweden), and Toronto (Canada). The results of this analysis are presented in Figure 1. They demonstrate that both techniques were equally capable of isolating sperm exhibiting high levels of total and progressive motility. DGC, which uses powerful centrifugal forces to isolate the highest density sperm, isolated significantly more sperm than Felix™. However, the latter was associated with a significantly higher level of vitality (Figure 1; $P < 0.001$). Importantly, the Felix™ System was also superior in generating populations of sperm exhibiting lower levels of DNA damage compared with both the original semen sample and the DGC populations. Moreover, the capacity of Felix™ to isolate sperm exhibiting low levels of DNA damage was demonstrated with two different DNA damage-monitoring techniques; SCSA (Figure 1E) and the HALO assay (Figure 1D).

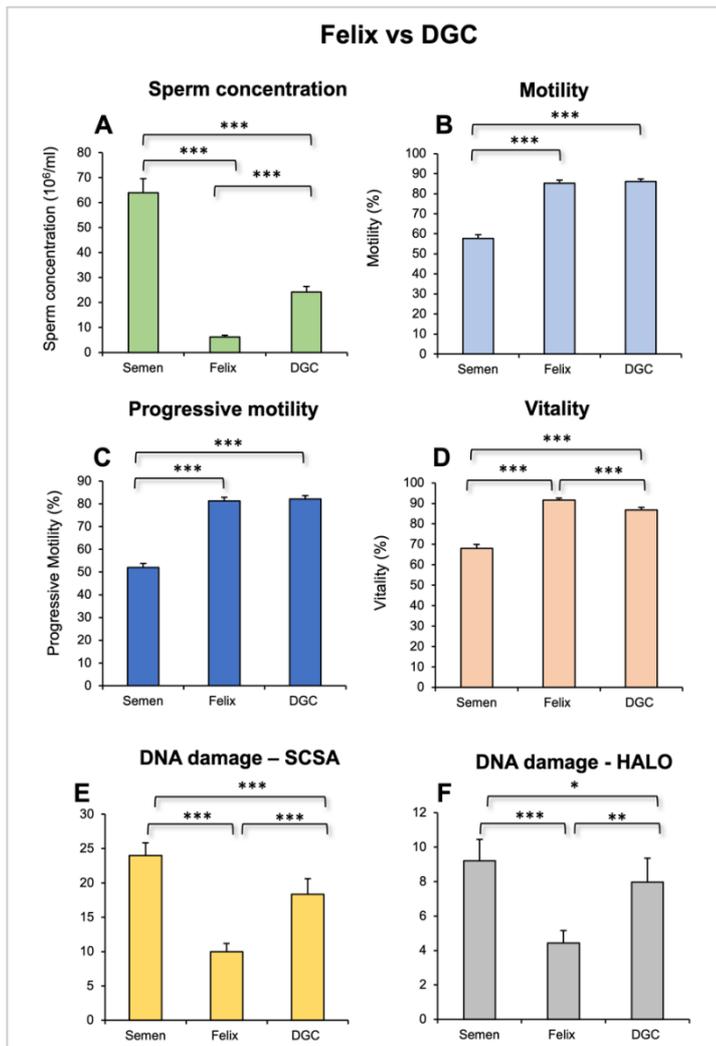


Figure 1:

Comparison between Felix™ and DGC for sperm isolation.

(A) sperm concentration, n=89;

(B) Motility, n=89;

(C) Progressive Motility, n=89;

(D) Vitality, n=54;

(E) DNA damage by SCSA, n=39;

(F) DNA damage by HALO,

(n=15. * P<0.05, ** P< 0.01;

*** P<0.001.)

In order to improve the quality of sperm generated by DGC, colleagues in the Reproduction Clinic, Osaka, Japan, prepared sperm cells using a two-phase technique comprising routine DGC followed by isolation of the most motile cells using a secondary swim-up procedure and compared the outcome of this protracted procedure with Felix™. The results of this analysis are presented in Figure 2. It is clear from these results, that if DGC is followed by a swim-up, then populations of sperm can be generated that have the same low levels of DNA damage as achieved with Felix™ but with significantly more motility. In this case, the major advantage of Felix™ was a considerable efficiency gain, requiring only 6 minutes of processing to achieve outcomes comparable with the double DGC-swim up technique, which takes at least 1 hour to perform (Yamanaka et al., 2016)

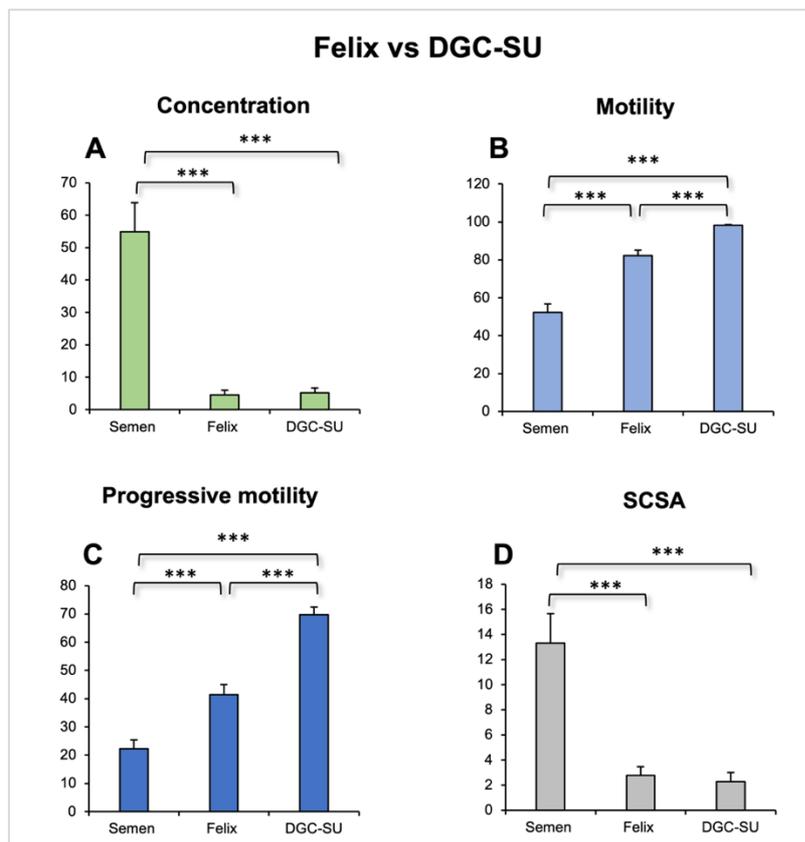


Figure 2:

Comparison between Felix™ and a two-phase DGC-swim-up approach to sperm isolation.

(A) Sperm concentration, n=25

(B) Motility, n=25

(C) Progressive Motility, n=25

(D) DNA damage by SCSA.

(n=24. * P<0.05, ** P< 0.01; *** P<0.001.)

We conclude from these *in vitro* studies that the Felix™ System is capable of isolating highly motile populations of sperm exhibiting low levels of DNA damage. Importantly, this system is significantly more effective than conventional DGC techniques and comparable with the double DGC-swim up procedure to isolate high quality sperm. However, the Felix™ System sperm is distinguished by its ability to isolate sperm for IVF/ICSI directly from semen in a matter of minutes. To our knowledge, currently there is no other sperm separation system in the market that can offer the same combination of speed and quality of isolated sperm as the Felix™ device.

Future *in vitro* studies will examine the relative capacity of this system to isolate sperm from seriously compromised samples (severely oligoasthenoteratozoospermic, cryopreserved semen, and testicular biopsy material) in comparison with DGC as well as other techniques that rely on the intrinsic motility of the sperm. Future studies will also evaluate the Felix™ System's performance in embryo and pregnancy studies.

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Both authors are employees of Memphasys Ltd, the developer of the Felix™ System and the financial sponsor of the research.

The Felix™ System is not included on the Australian Register of Therapeutic Goods (ARTG) and is not available for clinical use in Australia

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References

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