Review Article

Microfluidics in andrology

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Abstract

Male factor infertility is the sole cause of infertility in approximately 20% of the infertile couples with sperm playing a crucial role in the whole process of fertilization. Many technologies such as swim-up and density gradient techniques have been developed for the counting and sorting of sperms over the past few decades. Microfluidic technology was introduced into this field at the beginning of the 21st century. Studies have reported that conventional semen processing techniques have adverse effects on sperm deoxyribo nucleic acid (DNA) integrity leading to decreased pregnancy rates and are also associated with potential promutagenic alterations to sperm DNA. A gravity-driven microfluidic device, on the other hand, isolates motile sperms with good DNA integrity from seminal plasma, dead sperm, and debris, using microscale laminar flow without centrifugation. The objective of this review study is to evaluate whether microfluidic sperm sorters allow the effective recovery of sorted motile sperm without DNA damage compared with the centrifugation and swim-up procedure.

Keywords: Conventional swim-up procedure, density gradient centrifugation, microfluidics, sperm DNA fragmentation

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INTRODUCTION

About 30–50% of clinical infertility cases occur because of male factor with sperm defects.[1] Sperm parameters including extremely low sperm concentration, poor sperm motility, and abnormal morphology, hormonal disturbances, and physical and psychological problems may contribute to natural fertility problems. Earlier, these abnormalities were not completely overcome by contemporary infertility treatments such as intra uterine insemination and conventional in vitro fertilization (IVF), but the advent of intracytoplasmic sperm injection (ICSI) in early 1990s has allowed patients with oligozoospermia or azoospermia to father children.[2] ICSI enables the fertilization of an egg with one sperm selected from ejaculated spermatozoa. Therefore, sperm quality parameters, including motility, morphology, viability, DNA integrity, apoptosis, and maturity, are the critical determinants of successful assisted reproductive techniques (ART) outcomes.[3] Several methods such as double-density centrifugation and conventional swim-up procedure are performed for the isolation and the collection of the high-motility spermatozoa from semen samples and removing any impurities that interfere with fertilization.[4] However, several studies found that single or multiple centrifugation steps induce sperm DNA damage and the generation of reactive oxygen species (ROS)[5,6] producing adverse consequences during the post-implantation development of the embryo rather than before it.[7] Therefore, there is a need to develop sperm separation technique that facilitates the retrieval of spermatozoa with normal DNA integrity from ejaculated semen.

Access this article online

Quick Response Code:

Website: www.fertilityscienceresearch.org

DOI: 10.4103/fsr.fsr_4_18

How to cite this article: Talwar P, Yadav S. Microfluidics in andrology. Fertil Sci Res 2017;4:8-14.

SPERM PREPARATION TECHNIQUES

Different sperm preparation techniques are used to prepare spermatozoa for artificial insemination but the choice is strongly dependent on the quality of the semen. So, a prewash analysis of the semen is suggested to quantify the concentration, motility, and morphology of spermatozoa to obtain the higher number of motile spermatozoa even from the poor semen. The most common techniques of sperm preparation are swim-up technique and density gradient technique. Recent advances such as Microfluidics sperm sorting is also one of the sperm preparation techniques used to isolate highly motile spermatozoa with good integrity and will be described below:

(1) **Swim-up technique**

Swim-up is one of the most commonly used techniques for sperm preparation in IVF laboratories and is preferred if the semen sample has a normal number of good sperms (normozoospermia). Using this technique, sperms can be selected on the basis of their motility and the capability to swim out of the seminal plasma.[8] Swim-up can be performed using a cell pellet or a liquefied semen sample. In conventional swim-up, a liquefied semen sample is washed twice with sperm wash media and the pellet obtained by a soft spin is placed in an overlaying culture medium in a conical tube. The common steps of this technique are as follows:

(a) Allow specimen to liquefy completely for 15–30 min in an incubator at 37°C before processing.
(b) Perform a prewash analysis, that is, volume, sperm count, and motility.
(c) Gently mix the specimen with 4 ml of sperm wash media (human tubular fluid, HTF) using a sterile pipette.
(d) Centrifuge the tubes at 1500 rpm for 10 min.
(e) Carefully aspirate the supernatant without disturbing the pellet and resuspend the pellet in 2 ml of fresh HTF. Transfer the resuspended sample into a sterile round bottom tubes using a sterile pipette.
(f) Centrifuge the tubes at 1500 rpm for 5 min.
(g) Again carefully aspirate the entire supernatant from the round bottom tube using a pipette, with the tip placed just above the pellet surface.
(h) Resuspend the pellet in a 0.5 ml HTF using a 1 ml sterile pipette and incubate the tubes at a 45° angle for 20 min for swim-up in vertical rack in a 37°C incubator.
(i) Record the final volume and motility of the sperms.

(2) **Density gradient centrifugation (DGC) technique**

DGC separates sperm cells based on their density. It is preferred in the case of oligozoospermia, teratozoospermia, and asthenozoospermia.[8] Thus, at the end of centrifugation, each spermatozoon is located at the gradient level that matches its density. This method uses two gradients: a lower phase (90%) and an upper phase (45%), and sperm washing medium (modified HTF with 5.0 mg/ml human albumin) is used to wash and resuspend the final pellet. Below are some of the main steps of the process:

(a) Place all components of the 45 and 90% gradient and semen samples in an incubator at 37°C for 20 min.
(b) Transfer 1 ml of the lower phase into a sterile conical centrifuge tube.
(c) Layer 1 ml of the 45% gradient on top of the 90% gradient using a transfer pipette. Slowly dispense the upper phase lifting the pipette up the side of the tube as the level of the 45% gradient rises. A distinct line separating the two layers will be observed. This two-layer gradient is stable for up to 2 h.
(d) Measure semen volume to be loaded using a sterile 3 ml pipette. Remove a drop of semen for a prewash analysis.
(e) Gently place 1 ml of liquefied semen over the upper phase.
(f) Centrifuge for 20 min at 2000 rpm.
(g) Remove supernatant using a transfer pipette without disturbing the pellet, add 2 ml of HTF, and resuspend pellet. Mix gently with pipette until sperm pellet is in suspension.
(h) Transfer this suspension into a sterile round bottom tube and centrifuge for 10 min at 1500 rpm.
(i) Again, remove supernatant from the centrifuge tube using a transfer pipette down to the pellet.
(j) Resuspend the final pellet in a volume of 0.5 ml using a 1 ml sterile pipette with HTF and incubate the tubes for 20 min for swim-up. Record the final volume.

(3) **Microfluidic sperm sorter (MFSS)**

Microfluidic sperm sorters are used to isolate morphologically normal human spermatozoa with high motility and with no debris based on fluid dynamics in a microenvironment or nanoenvironment, relying on variables such as fluid density, viscosity, velocity, and size/geometry of the environment.[9] Microfluidics have been widely applied to biomedical fields, because the control of fluid transport is useful for

cell analysis systems, drug delivery systems, assisted reproductive technologies, and in numerous biological applications specifically for the miniaturization and simplification of laboratory techniques.[10] Sperm selection in this method is based on the two gravity-driven laminar flows within the central microfluidic channel. The semen and the medium dispensed into inlet A and B, respectively, flow parallel to each other and exit through their respective outlets (A/D and B/C). Spermatozoa are sorted depending on their ability to swim across these two streams. Only motile spermatozoa swim toward outlet C whereas immotile spermatozoa keep flowing to outlet D.[11] Hence, this method does not allow spermatozoa to undergo added physical stress from sources such as a centrifuge and thus minimize DNA damage.

Among the sperm sorting via microfluidic devices, Horsman *et al.*[12] sorted motile spermatozoa based on their ability to move out of their initial streamlines in the laminar fluid flow using a microfabricated device. Cho *et al.*[13] put forward a horizontally set up gravity-driven pumping system to simplify the sorting step without centrifugation step to separate 100% motile sperms. Nowadays, there are some innovative sperm separation devices that have been developed. For example, Lin *et al.*[14] suggested a microfluidic device with diffuser chamber, which separates the sperm by the velocity gradient. Ainsworth *et al.*[15] proposed a rapid and safe method to isolate the motile human spermatozoa with less DNA damage by size and electric field using an electrophoretic system. In particular, the channels and reservoirs were coated with 1% bovine serum albumin to improve the surface hydrophilicity.[16] By varying the channel dimensions, numerical simulations were applied to confirm the separation efficiency of sperm sorter. The device isolates motile sperm from immotile sperm and other cellular debris.[17]

(a) **Media flow in ART microfluidic devices**

Early devices used for ART applications have employed gravity-driven passive flow, with hydrostatic pressure in media reservoirs as an important variable to drive media flow through microchannels.[16] Many have utilized manually applied pressure via externally attached syringes to input/output ports to cause media flow through microfluidic devices. Later on, the use of Hamilton syringes attached to a programmable infusion pump has been adapted.[18] In addition, one study showed the use of a tilting culture system in conjunction with microfluidic channels using gravity, which offered more consistent control over flow. Finally, a Braille pumping system using tiny electric piezo-actuators has been used successfully to peristaltically move media along microchannels during embryo culture, while the embryos remain largely undisturbed.[19]

(b) **Fabrication of microfluidic device**

Microfluidic channels are fabricated by a standard soft lithography technique using poly-dimethylsiloxane (PDMS)[16] silicone, borosilicate glass, Pyrex, quartz, or the combinations of these materials. Construction materials for microfluidic channels may vary from materials selected for cell substrate. Microchannels are manufactured by techniques involving molding, photolithography, and chemically or mechanically etching channels into suitable materials. PDMS polymer is the most commonly used material for microchannel fabrication, which is selected due to its inherent use and fabrication advantages, such as flexibility, ease of soft-lithography patterning, and low autofluorescence for use with microscopy.[17]

(c) **Procedure**

Microfluidic sperm sorter commonly used in our laboratory is Sperm Sorter QUALIS which is a cyclo-olefin polymer-based MFSS chip manufactured by Menicon Co. Ltd. It has four chambers named chamber A, B, C, and D and microchannels being connected to each other [Figure 1]. The common steps of this method are as follows:

(i) Before sperm separation, semen samples were filtered using a 20 mm pore size filter to remove foreign bodies (optional).

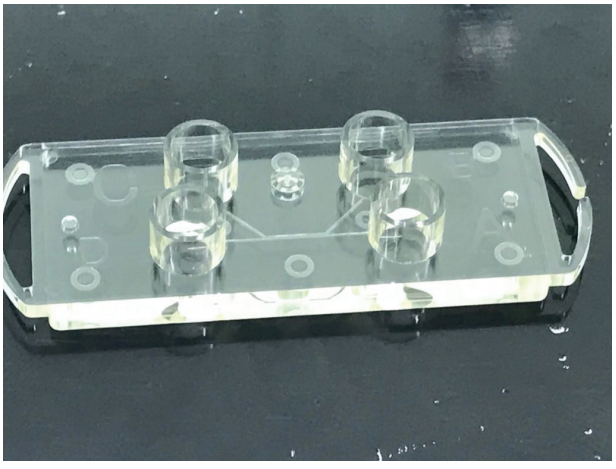

Figure 1: Microfluidic sperm sorter having four chambers, named chamber A, B, C, and D, and microchannels being connected to each other

(ii) Each ejaculated sample was carefully diluted at 1:1 ratio with sperm sorting medium (G-MOPS™ PLUS supplemented with HAS, manufactured by Vitrolife Sweden AB V. Frölunda, Sweden (Ref.: 10130)) and maintained at approximately 37°C before using the MFSS.
(iii) Then the MFSS was fixed in a 60-mm dish.
(iv) After fixing of the MFSS, 100 µl of the sperm-sorting medium was loaded in chambers A, B, C, and D to create streamlines in the microfluidic channels and then the medium was pulled out from all chambers.
(v) Twenty microliters of the medium was loaded in chambers C and D, 100 µl of the medium was loaded in chamber B, and 65 µl of sperm suspension was loaded in chamber A.
(vi) The amount of medium in chamber B was adjusted such that the width of the laminar flow from chamber A was approximately 40% of the overall width of the central microchannel under the microscope [Figure 2].
(vii) After 20 min, 20 µl of completely isolated motile spermatozoa were extracted from chamber C and used only for ICSI.

Review of literature

This paper has presented a systematic review of the effects of microfluidics on sperm motility, morphology, and integrity over the past 20 years since the idea of microfluidics was developed two decades back. To start with, we searched for the articles related to keywords such as microfluidics, sperm DNA fragmentation (SDF), and DGC on the PubMed site of National Center of Biotechnology Information and selected five articles whose detailed description is provided in Table 1.

Figure 2: The amount of medium in chamber B was adjusted such that the width of the laminar flow from chamber A was approximately 40% of the overall width of the central microchannel

Schulte *et al.*[20] in their article reported that semen samples prepared by microfluidics have reduced the percentage of fragmented DNA and increased the motility when compared to unprocessed semen, serial centrifugation, swim-up, and DGC. Shirota *et al.*[21] in 2016 reported that low SDF was detected in the case of MFSS preparation than centrifugation and swim-up procedures. Another paper by Wang *et al.*[22] in 2011 reported that sperm motility, sperm morphology, SDF, and tail hypo-osmotic swelling rate were significantly improved in microfluidic sorted sperms. Furthermore, Kishi *et al.*[7] reported that the rate of SDF was lower in samples sorted by MFSS than the swim-up preparations. Moreover, Schuster *et al.*[23] analyzed the increased sperm motility and morphology in MFSS preparations.

Reproductive outcomes after using MFSS

Ramakrishnan *et al.*[24] presented a study via poster on sperm preparation by MFSS − comparison of DNA fragmentation index, embryo quality, clinical pregnancy rate, and implantation rates with DGC at 33rd Annual Meeting of ESHRE, Geneva, Switzerland. It included 66 men recruited for IVF/ICSI whose semen samples were prepared by DGC and MFSS technique. These preparations along with prewash sample were assessed for SDF using sperm chromatin dispersion test. Oocytes collected from the respective partners were equally divided into injected with sperms sorted by DGC and MFSS. Fertilization rates and embryo quality were recorded on Day 3. Pregnancy rates and implantation rates were also computed.

Results showed that SDF rates were reduced from 36 ± 12.85% in prewash samples to 15 ± 11.10% in DGC group and 9.66 ± 7.89% in MFSS group. In addition, an increase in fertilization rates (74.03% vs. 79.83%) and embryo quality (59% vs. 63%) was noticed for DGC group and MFSS group, respectively. Moreover, MFSS resulted in 83.33% pregnancies when compared to 75% in DGC, whereas implantation rates were 42.10% in DGC and 45.70% in MFSS.

Table 1: Various studies showing the effect of microfluidics on sperm integrity

S. No.	Article title, authors and year	Type of study	Description of study	Results
1.	Microfluidic sperm sorting device provides a novel method for selecting motile sperm with higher DNA integrity.Schulte *et al*. (2007)[20]	Experimental laboratory study	Ten semen samples were obtained from men presenting for infertility evaluation. Samples were individually divided into five aliquots for subsequent analysis of sperm motility and DNA integrity: (i) Unprocessed semen; (ii) Serial centrifugation; (iii) DGC; (iv) Swim-up; (v) Microfluidic sperm sorting. Semen samples were assessed for DNA integrity using sperm chromatin dispersion and expressed as percentage of sperm with fragmented DNA.	The percentage of sperm with fragmented DNA was reduced significantly with microfluids (1.9%) compared to unprocessed semen (13.3%), serial centrifugation (15.8%), DGC (14.9%), and swim-up (5.7%). Mean sperm motility improved significantly with MFSS (96.2%) compared to unprocessed semen (52.0%), serial centrifugation (50.1%), DGC (73.4%), and swim-up (85.8%).
2.	Separation efficiency of a MFSS to minimize sperm DNA damage.Shirota *et al*. (2016)[21]	Experimental laboratory study	Semen samples from 37 healthy volunteers were collected and were divided in three groups: (i) Swim-up; (ii) Centrifugation; (iii) Microfluidic sperm sorting. DNA damage after sperm preparation using MFSS, centrifugation, and swim-up procedures were assessed using flow cytometric measurements and sperm chromatin structure assay.	Final sperm concentration and motility were significantly different between the centrifugation and swim-up procedures and MFSS sperm preparations. Lower sperm DNA fragmentation rate was detected in MFSS preparations compared with centrifugation and swim-up procedures.
3.	Effects of a MFSS on sperm routine parameters and DNA integrity.Wang *et al*. (2011)[22]	Case series	Forty semen samples were collected and divided into two aliquots: (i) Sperm sorting using a self-made polydimethylsiloxane MFSS; (ii) Swim-up method; Effect of these two methods on the sperm routine parameters and DNA integrity were evaluated and compared using computer assisted sperm analysis and sperm chromatin dispersion test.	Sperm motility, sperm morphology, and tail hypo-osmotic swelling rate were significantly improved in microfluidic sorted sperms. Microfluidic sperm sorter had a significantly lower rate of sperm DNA damage (8.4 ± 5.8%) than the swim-up method (6.6 ± 9.2%).
4.	Frequency of sperm DNA fragmentation according to selection method: Comparison and relevance of a microfluidic device and a swim-up procedure.Kishi *et al*. (2015)[7]	Original article	Semen samples from ten men with normal, oligozoospermia, and asthenozoospermia were split into two groups and sorted using a microfluidic device or by a swim-up method. Semen parameters and sperm DNA fragmentation were measured and analyzed using paired or nonpaired Student's *t*-tests.	Sperm DNA fragmentation decreased to 8.3% after swim-up preparation and to 5.9% in samples sorted by the sperm sorter. Similar findings were evident when DNA fragmentation of normal individuals was compared with a low motility group and a low sperm count group.
5.	Isolation of motile spermatozoa from semen samples using microfluidics.	Original article	Five motile sperm samples were prepared with density gradient separation.	There was no difference in sperm motility when compared with unexposed aliquots.

(*Continued*)

Table 1: (*Continued*)

S. No.	Article title, authors and year	Type of study	Description of study	Results
	Schuster *et al*. (2003)[23]		Sperm motility was assessed the following day after exposing aliquots to polydimethylsiloxane used to construct the device. Ten unprocessed semen samples were placed in wider channels and sperm motility and strict morphology were assessed from sorted outlets. Finally, six density gradient prepared samples containing 5 × 10^6 motile spermatozoa/ml and 50 × 10^6 round immature germ cells/ml were sorted and assessed in a similar fashion.	Sperm motility increased from 44 ± 4.5% to 98 ± 0.4% and morphology increased from 10 ± 1.05% to 22 ± 3.3% following processing. The ratio of motile spermatozoa to round immature germ cells in the wide inlet (1:10) was significantly improved in the thin outlet (33:1).

DGC = density gradient centrifugation, MFSS = microfluidic sperm sorter.

CONCLUSION

Selection of the best spermatozoa and elimination of damaged spermatozoa are critical for successful IVF and ICSI in infertility clinics. The most prevalent methods for the isolation of motile spermatozoa are density gradient separation and swim-up methods. However, one or two centrifugation steps are required to separate spermatozoa in both methods and this might cause damage to sperm integrity.[25] Furthermore, sperm damage by centrifugation might lead to increased levels of ROS causing SDF[26] that can affect the viable pregnancy and normal embryonic development. In addition, to perform the transfer of male genes in the oocyte during fertilization, sperms should have properly packed DNA in the nucleus to achieve a viable pregnancy.[27] Therefore, MFSS is the most commonly used device for sorting highly motile spermatozoa with less DNA integrity damage without subjecting sperms to undergo repeated centrifugation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP. Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 2003;80:895-902.
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17-8.
3. Brincat D, Catania S, Wismayer PS, Calleja-Agius J. Male factors in ART outcome prediction. Gynecol Endocrinol 2015; 31:169-75.
4. Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev 2007;CD004507.
5. Malvezzi H, Sharma R, Agarwal A, Abuzenadah AM, Abu-Elmagd M. Sperm quality after density gradient centrifugation with three commercially available media: A controlled trial. Reprod Biol Endocrinol 2014;12:121.
6. Iwasaki A, Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil Steril 1993;57:409-16.
7. Kishi K, Ogata H, Ogata S, Mizusawa Y, Okamoto E, Matsumoto Y, *et al*. Frequency of sperm DNA fragmentation according to selection method: Comparison and relevance of a microfluidic device and a swim-up procedure. J Clin Diagn Res 2015;9:QC14-6.
8. Natali I. Sperm Preparation Techniques for Artificial Insemination – Comparison of Sperm Washing, Swim-Up and Density Gradient Centrifugation Methods. Intech; 2011.
9. Beebe D, Wheeler M, Zeringue H, Walters E, Raty S. Microfluidic technology for assisted reproduction. Theriogenology 2002;57:125-35.
10. Suh RS, Zhu X, Phadke N, Ohl DA, Takayama S, Smith GD. IVF within microfluidic channels requires lower total numbers and lower concentrations of sperm. Hum Reprod 2006;21:477-83.
11. Matsuura K, Takenami M, Kuroda Y, Hyakutake T, Yanase S, Naruse K. Screening of sperm velocity by fluid mechanical characteristics of a cycloolefin polymer microfluidic sperm-sorting device. Reprod Biomed Online 2012;24:109-15.
12. Horsman KM, Barker SL, Ferrance JP, Forrest KA, Koen KA, Landers JP. Separation of sperm and epithelial cells in a microfabricated device: Potential application to forensic analysis of sexual assault evidence. Anal Chem 2005;77:742-9.
13. Cho BS, Schuster T, Zhu X, Chang D. A microfluidic device for separating motile sperm from nonmotile sperm via inter-streamline crossings. 2nd Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine & Biology, 2002.
14. Lin YN, Chen PC, Wu RG, Pan LI. Micro diffuser-type movement inversion sorter for high-efficient sperm sorting. 8th IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS), 2013, p. 1058-61.
15. Ainsworth C, Nixon B, Aitken RJ. Development of a novel electrophoretic system for the isolation of human spermatozoa. Hum Reprod 2005;20:2261-70.
16. Cho BS, Schuster TG, Zhu X, Chang D, Smith GD, Takayama S. Passively driven integrated microfluidic system for separation of motile sperm. Anal Chem 2003;75:1671-5.
17. Hyakutake T, Hashimoto Y, Yanase S, Matsuura K, Naruse K. Application of a numerical simulation to improve the separation efficiency of a sperm sorter. Biomed Microdevices 2009;11:25-33.

18. Hickman DL, Beebe DJ, Rodriguez-Zas SL, Wheeler MB. Comparison of static and dynamic medium environments for culturing of pre-implantation mouse embryos. Comp Med 2002;52:122-6.
19. Cabrera LM, Heo YS, Ding J, Takayama S, Smith DG. Improved blastocyst development with microfluidics and Braille pin actuator enabled dynamic culture. Fertil Steril 2006;87:S43.
20. Schulte RT, Chung YK, Ohl DA, Takayama S, Smith GD. Microfluidic sperm sorting device provides a novel method for selecting motile sperm with higher DNA intgerity. Fertil Steril 2007;88:S76.
21. Shirota K, Yotsumoto F, Itoh H, Obama H, Hidaka N, Nakajima K, *et al*. Separation efficiency of a microfluidic sperm sorter to minimize sperm DNA damage. Fertil Steril 2016;105:315-21.
22. Wang W, Liang GT, Peng YY, Liu DY, Zhou XM. Effects of a microfluidic sperm sorter on sperm routine parameters and DNA integrity. Zhonghua Nan Ke Xue 2011;17:301-4.
23. Schuster TG, Cho B, Keller LM, Takayama S, Smith GD. Isolation of motile spermatozoa from semen samples using microfluidics. RBMO 2003;7:75-81.
24. Ramakrishnan S, Balasubramanyam S, Amita S, Kumar P. Sperm preparation by microfluidic sperm sorter (MFSS) − Comparison of DNA fragmentation index, embryo quality, clinical pregnancy rate and implantation rates with density gradient centrifugation. 33rd Annual Meeting of ESHRE, Geneva, Switzerland, July 2-5, 2017.
25. Alvarez JG, Lasso JL, Blasco L, Nunez RC, Heyner S, Caballero PP, *et al*. Centrifugation of human spermatozoa induces sublethal damage; separation of human spermatozoa from seminal plasma by a dextran swim-up procedure without centrifugation extends their motile lifetime. Hum Reprod 1993;8:1087-92.
26. Muratori M, Maggi M, Spinelli S, Filimberti E, Forti G, Baldi E. Spontaneous DNA fragmentation in swim-up selected human spermatozoa during long term incubation. J Androl 2003;24:253-62.
27. Talwar P, Hayanagarkar S. Sperm function test. J Hum Reprod Sci 2015; 8: 61–9.