RESEARCH
O&G Forum 2024; 34 - 3s: 3022-3025

MICROFLUIDICS IN ASSISTED REPRODUCTION: ADVANCEMENTS IN SPERM SORTING FOR ENHANCED IVF OUTCOMES

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Abstract
Various advancements in assisted reproductive technology have enabled a safe and effective practice of In vitro fertilization. The success of IVF is subjected to the quality of both male and female gametes. While the quality of oocytes is highly dependent on age; sperm selection becomes a critical factor for a healthy embryo development for IVF. The conventional methods for preparation of sperms for IVF may provide sperms with sub-optimal quality due to DNA fragmentation. Microfluidics based sperm sorting devices provides an effective way to sort out sperms with high quality to be used in IVF. The device is based on the differential fluid mechanics of motile and non-motile sperms due to a contrast in their densities. Several comparative studies have indicated the advantages of microfluidics-based devices over conventional techniques in preserving the DNA integrity of sperms. It is also advantageous with respect to providing a streamlined clinical procedure, reducing the turn-over time for the preparation of sperm samples. This review provides an insight on the basic factors affecting the outcomes of IVF and application of microfluidic device in sperm selection.
Keywords: Microfluidics technology, Assisted reproduction, Sperm motility, Sperm DNA integrity, Density gradient centrifugation, Sperm selection techniques.

INTRODUCTION

Use of microfluidics technology in assisted reproduction

Different techniques to handle male fertility have been developed over years. In 1992, the most successful technique to facilitate targeted fertilization was devised by microinjecting a single sperm inside the oocyte. The technique was named as intracytoplasmic sperm injection (ICSI) [1]. ICSI was initially used only in the case of low sperm count or low sperm mobility leading to failure in fertilization of oocyte but has become the commonly used method of fertilization due to its high success rate. Fertilization is carried out *in vitro* by culturing a single oocyte with 50-100 thousand sperms. Sperms are prepared for fertilization by separation by density centrifugation and washing in a media with high protein concentration for capacitation. Conversely, ICSI may be used for the fertilization of oocytes, especially in the cases of male factor infertility [2, 3].

The selection of high-quality sperms is an essential factor for the success of IVF procedure and the health of offspring conceived using IVF. Currently, the most widely used methods for sperm selection includes density gradient centrifugation and swim-up methods. However, both these methods can potentially affect the quality of sperms and may lead to the transmission of genetic defects due to compromised sperm DNA integrity. Microfluidics based devices have been employed as an alternate sperm selection technique with an advantage of high throughput, sensitivity, portability and automation with minimum effect on sperm DNA integrity. Several different microfluidics platforms have been designed with confined microchannels, porous membranes, periodic micropillar arrays or laminar flow streams [4-10]. Microfluidics technology is not only limited to sperm sorting but can be utilized in other aspects of ART such as infertility diagnosis, sperm guidance, insemination, oocyte analysis, embryo selection and cryopreservation[11, 12]. Sperm sorting by using microfluidics device is based on differential sperm motility to enable selection of highly motile sperms for IVF. The sperms processed by microfluidics device are subjected to minimal oxidative stress and therefore lesser DNA damage as compared to conventional methods of swim-up and density gradient centrifugation [4, 10, 13].The geometrically confined environment of microfluidics device mimics the natural environment of reproductive canal. Thus, the medium used for sperm sorting in microfluidic devices should have similar viscosity as cervical mucus in reproductory tract. Hyaluronic acid (HA) and methyl cellulose (MC) based media are often used for this purpose [14, 15].

Emergence of microfluidics technology has enabled efficient outcomes in different fields of science. It has proved to be highly beneficial in the optimization of sperm sample preparation to be used in IVF. Microfluidics based sperm sorting devices are capable of processing small sample volumes and reduces the cost of sperm processing by other less effective methods.

Additionally, it has an advantage of high throughput sample processing with low response time and high sensitivity. Selection of healthy sperms is a critical determinant in the success of IVF. Microfluidics based sperm sorting devices enables the recovery of motile sperms from a heterogenous population in a semen sample, thus reducing the additional steps and time required to enrich the sperm sample for IVF.

Factors influencing the outcomes of IVF

Infertility is a fairly common problem in today's era with the prevalence of various etiological factors leading to either male or female infertility [16]. It is estimated that approximately 15% of couples face problems with conceiving childbirth [17]. In-vitro fertilization is considered as the most common and most successful form of ART and has proved to be a highly beneficial technique in infertility management. Despite being the most successful form of ART, the outcome of IVF is dependent on various factors. The efficiency of IVF cycle is based on the frequency of live birth per embryo transfer [18]. The global success rate of IVF is around 40% in young women. Considerable improvement in the success rate of IVF has been observed after the development of advanced and innovative procedures to aid in the infertility treatment [19]. Still, intrinsic factors play a major role in the result of IVF and being a multi-step procedure, its outcome requires the robustness of each step. Among the factors which influence the outcome of IVF, age is the most critical. Fertility of women declines as age progress and so does the likelihood of pregnancy after 35 years of age using IVF [20]. Couples need to undergo standard infertility evaluation before starting IVF. This includes examination of female reproductive tract and analysis of sperm in terms of motility and number of sperms per ejaculation [21, 22].Reduction in the ovarian reserve due to reproductive tract disorders like endometriosis or primary ovarian insufficiency (POI) usually require oocytes from a donor. Also, the couples having a successful pregnancy in the past have higher chances of success for IVF while a history of miscarriages decreases this chance [23]. The quality of oocyte, sperm and embryo used for transplantation is a vital determinant for successful live birth through IVF. The protocol utilized for ovarian stimulation during an IVF cycle affects the quality of oocytes as well as resulting embryo after fertilization [24]. Therefore, utmost care and precautions must be taken while choosing the method for ovarian stimulation. Poor quality of semen is an etiological factor for infertility. Suboptimal sperm quality includes decrease in sperm count, presence of higher numbers of immotile sperms or higher fraction of sperms with aberrant morphology (abnormally shaped sperm head, two headed sperms, sperms without tail etc) [25]. Sperm DNA integrity is another reason for infertility which affects embryo development [26]. Conventional methods for IVF may not be effective in case of poor sperm quality and would require methods like ICSI for targeted fertilization. Overall, there are multiple challenges in each step of IVF which can be overcome by careful assessment of every step as well as utilizing advanced techniques in practice.

Working of microfluidics devices in sorting of healthy sperms

Microfluidics technology is utilized for cell sorting based on the study of liquid behaviour at microliters level [27]. The liquid under investigation is passed through microchannels and processed to separate the sample from the waste. The technology has its application in the field of all three branches of science *i.e.,* physics, biology and chemistry [28]. The potential of microfluidics has been recognized in ART, where it is used to sort out sperms on the basis of motility and morphology [29]. Isolation of high-quality spermatozoa is desirable for the success of IVF procedure. The semen sample is processed by the technician before fertilization to obtain high quality sperms. This includes combination of processes such as washing sperm with high protein media for capacitation, sperm swim up out of the seminal fluid and density gradient centrifugation [30].Additionally, introduction of microfluidics technology has been known to improve the overall content of quality sperms [31]. Semen sample processed by microfluidics contains nearly 98% motile sperms as compared to 44% motile sperms in unprocessed sample [32]. Also, there an 80% improvement in DNA integrity of sperms while conventional process like density gradient centrifugation may lead to DNA damage. Another advantage of this technique is that a low volume of semen can be processed by microfluidics procedure. Generally, a microfluidics sperm sorting device consists of channels with two inlets for buffer and sample while two outlets for sorted sperms and waste in the form of seminal fluid, debris and poor-quality sperms (Fig 1). The flow of sample is maintained by a passive pumping system generated by the height difference between inlet and outlet reservoir [33]. The process of cell sorting is based on the segregation of cells of different size and density experiencing different inertial force, when forced through a narrow spiral microchannel or straight channel towards the collection chamber based on the design of the device [7, 34].The denser cells migrate through the outer rim of channel while the less dense cells tend to move through the center of the channel.

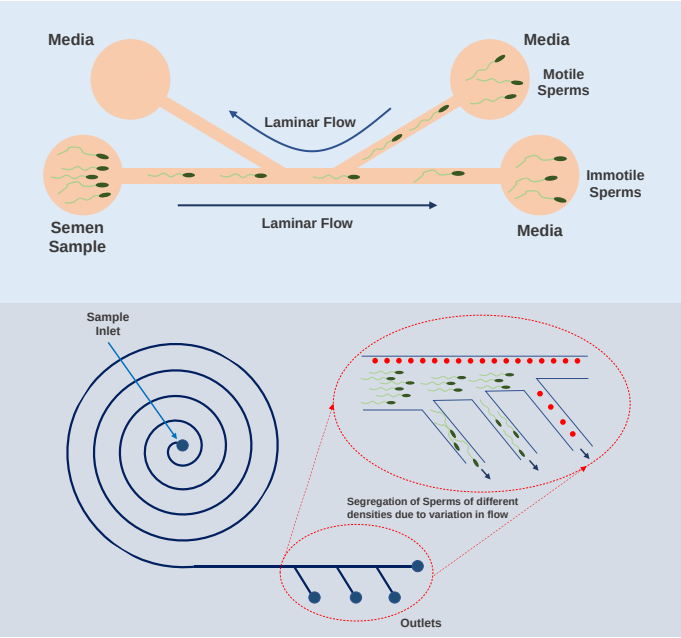

Fig 1. Sorting and segregation of motile sperms from motile sperms in a semen sample using microfluidics device. (Upper panel: Microfluidics Sperm Sorting Qualis, Lower Panel: Microfluidics Sperm Sorting device with spiral channels).

Significance and advantages of microfluidics device in sperm sorting

Success of IVF is dependent upon the efficacy of technical procedures as well as the quality of gametes used for fertilization. Thus, it is critical to select the best quality spermatozoa from a pool of sub-optimal quality sperms. The most common method for the isolation of good quality sperm is density gradient centrifugation and swim-up method. However,

both the methods have its disadvantages in terms of efficacy as well as downgrading the quality of sperms [35-37]. Density gradient centrifugation result in an increase in the level of reactive oxygen species (ROS) leading to sperm DNA fragmentation (SDF) due to oxidative damage[38, 39].Also, the handling time between the centrifugation steps affects the viability of the sperm resulting in lesser sperm subpopulation with high DNA integrity [38-40]. DNA damage may result in abortive apoptosis and defective maturation of sperms [41]. Adverse effects of SDF have been observed in the development of embryo in the post-implantation phase [42]. SDF is associated with poor blastulation and increased instances of pregnancy loss. There are techniques to detect SDF in sperms such as sperm chromatin structure assay (SCSA) and terminal deoxynucleotidyl mediated fluorescein-dUTP nick end labeling (TUNEL) [41, 43]. The results of these techniques are often inconsistent and it is more desirable to avoid selection of sperms with DNA damage rather than detecting the damage for the sake of cost effectiveness.

Use of microfluidics-based device for the sorting of sperm having high motility on the basis of differential flow properties due to variation in density [7]. Microfluidics technology has an advantage over other techniques in causing minimal stress to sperms and thus lesser SDF [37, 44].Belloc *et al.,* established the cut-off value for the content of SDF to be >30%,on the basis of 1974 normozoospermic semen sample and 4345 semen samples from infertile men [45]. Another study by Kishi *et al.,* on the comparison of SDF in the semen samples processed by density gradient centrifugation and Microfluidics-based device showed the effectiveness of the latter in minimizing the DNA damage. The initial unprocessed semen samples had 27.7% of SDF while there was 25.8% of SDF in semen sample after density gradient centrifugation. The SDF was decreased significantly up to 8.3% after processing with microfluidics-based device [37]. Pujol *et al.,* analyzed semen samples for DNA fragmentation by swim-up technique and a microfluidic sperm sorting device and found a significant difference in the extent of DNA damage. The SDF measured was 64.8% for unprocessed semen sample, 65.1% for sample processed by swim-up technique while it reduced to a significant level of 34.9% when processed by microfluidic sperm sorting device [46]. Similar study was carried out by Quinn *et al.,* in which DNA fragmentation index (DFI) was calculated for semen samples processed by density gradient centrifugation, swim-up technique and microfluidic chip device. Semen samples processed by microfluidic chip device showed lower DFI as compared to the other two techniques [13]. The viability of the sperm is also dependent upon the swimming media. Eamer *et al.,* studied the effect of hyaluronic acid (HA) and methyl cellulose (MC) based high viscosity media on sperm viability using microfluidic device. It was found that there was 274% increase in sperm viability using MC media while HA media resulted in a sperm subpopulation with 133% increase in viability [47]. There are various advancements in working designs available for microfluidics-based sperm sorting device aiding to the robustness and efficacy in sperm sorting for IVF [11]. Simchi *et al.,* designed a device with 6500 parallel channels to isolate sperms with DNA integrity up to 95% [48].

CONCLUSION

Since the advent of IVF, several advancements have occurred in the field of assisted reproductive technologies. Although, IVF has a high success rate in younger couples with healthy gametes, there is a decline in the rate of successful outcomes if the sperms are sub-optimal. Density gradient centrifugation and swim up method are conventional methods to isolate sperms from the seminal fluid. These methods are not effective in the selection of high-quality sperms due to compromise in DNA integrity. Microfluidics based device provides a suitable option for sorting sperm subpopulation on the basis of motility having high DNA integrity. Microfluidics devices of different working designs showed their efficacy in providing sperms with minimal DNA fragmentation which is critical to a positive outcome in IVF.

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