

SPERM PREPARATION - SWIM UP, DENSITY GRADIENT, MICROFLUIDIC SPERM SORTER METHOD & MAGNETICACTIVATED CELL SORTING METHOD - A COMPARATIVE ANALYSIS

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DOI: 10.47750/pnr.2022.13.501.63

Abstract

Infertility is a major concern for many couples who want children of their own. The cause of infertility may be with either of the partners. The use of Assisted Reproductive Techniques (ART) is the solution for those couples who have infertility issues. In this article, the main focus is on male infertility. In order to determine the acceptability of processed semen for breeding purposes, a precise evaluation of semen fertilizing capacity is critical. A solid sperm preparation process yields a sample with excellent viability and motility, as well as taking into account other factors like Capacitation and apoptosis, which can affect the capacity to fertilize an oocyte. In order to determine the acceptability of processed semen for breeding purposes, a precise evaluation of semen fertilizing capacity is critical. A solid sperm preparation process yields a sample with excellent viability and motility, as well as taking into account other factors like Capacitation and apoptosis, which can affect the capacity to fertilize an oocyte. This article provides a comparison of four different sperm processing/washing techniques of swim up, density gradient, microfluidic cell sorter method & magnetically activated cell sorting method. The various aspects of each of these techniques are discussed.

Keywords: Swim Up, Density Gradient, Microfluidic & Magnetic Activated cell sorting Method.

I. INTRODUCTION

Male factors are responsible for about half of all infertility cases. Assisted reproductive technologies (ARTs) can help these patients overcome their spermatozoa's inability to fertilize the egg and produce viable and healthy offspring, but the effectiveness of the various procedures can still be improved. According to the European Society of Human Reproduction and Embryology (ESHRE) and the Centres for Disease Control and Prevention of the United States (CDC), the percentages of deliveries per ART cycle were 21 and 22 percent in 2014 and 2016, respectively. The quality of the spermatozoa has been cited as one of the causes of the low efficiency, and the existence of high percentages of DNA-damaged spermatozoa in patients' ejaculates is potentially one of the key factors lowering ART outcomes. As a result, one of the major problems in reproductive medicine is to ensure that the spermatozoa utilized in ARTs are of the greatest quality, particularly in terms of genetic integrity [1].

In recent decades, ART has been regarded as the primary treatment for both males and females suffering from infertility. Sperm quality has a significant impact on the success of ART treatment [2]. In the last two decades, a range of strategies for selecting high-quality sperm for fertilizing eggs for ART has been developed, and those procedures have had a direct impact on the results of ART [3]. In order to go into the ART process, it is essential to prepare the sperm accordingly. Of the many steps involved, sperm washing is one of the primary steps.

Sperm washing is a type of sperm preparation that is required prior to intrauterine insemination (IVF) because it removes toxins from the sperm that could induce negative uterine reactions. Sperm is isolated from seminal fluid during the sperm washing procedure. In cases of immune system problems, male-factor infertility, or unexplained infertility, this technique improves the fertilizing potential of the sperm. The different methods involved are discussed in the upcoming sections.

II. SPERM PREPARATION METHODS:

In order to integrate into the routines of human clinics, sperm selection/preparation processes should be operatively easy and cost-effective. They must also ensure that the sample is enriched in high-quality spermatozoa in the shortest time possible. In addition to removing low-quality spermatozoa, such as those that are immotile, sperm preparation techniques should allow for the removal of other cells such as leukocytes and bacteria, as well as harmful or bioactive chemicals such as reactive oxygen species (ROS) [3].

Spermatozoa exposed to seminal plasma for an extended period of time have been reported to have negative impacts on sperm function. Despite the fact that seminal plasma protects spermatozoa from certain detrimental situations including oxidative stress [4], ejaculated semen contains senescent sperm and particle debris, which might limit sperm survival. As a result, mammalian spermatozoa are frequently separated from seminal plasma in the laboratory and in assisted reproductive technology applications. This procedure is known as "sperm washing," and it involves removing spermatozoa from seminal plasma and resuspending them in a culture medium. There are various methods that can be used in sperm preparation techniques, each having its own advantage. For the purpose of this review, the different methods discussed here are Swim Up, Density Gradient, Microfluidic cell sorter method & Magnetic Activated cell sorting Method.

III. Swim Up (SU) Method:

Mahadevan and Baker (1984) [5] were the first to describe SU, which is based on the recovery of motile spermatozoa that move toward a cells-free media that is normally placed above the sperm sample. Centrifugation [5], straight migration from unprocessed semen [6], recovery of spermatozoa from non-resuspended pellet [7], or spermatozoa sedimentation by gravity prior to SU [migration sedimentation (MS) method] are some of the SU methods available [8].

The SU is the most popular method used in ART labs, and it is chosen if the sperm sample has a normal quantity of healthy sperm (normozoospermia). Sperms are chosen based on their motility and ability to swim out of the seminal plasma using this method.

The most basic approach for sperm cell preparation is known as the swim-up (Figure 1). To extract the seminal fluid, the sample is centrifuged with a sperm wash medium and then placed in an incubator at 37 °C with a 45° inclination and an overlapping layer of medium for 60 minutes. The technique's idea is based on sperm cells being drawn to the most enriching part of the liquid. Cells with progressive motility can rise from the pellet via the medium and be recovered from the surface under these circumstances, promoting Capacitation and migration [5]. There are also various versions of the approach that eliminate the need for centrifugation by allowing sperm cells to flow straight from the seminal fluid to the medium surface, which is simply placed over the sample [9]

SWIM UP METHOD





DG METHOD:



Figure1. SU method & DGC method [13]

This procedure is used for ART, and it is advised when the percentage of motile spermatozoa in the sample is low [9]. Despite the fact that it involves centrifugation, swim-up is a gentle approach that creates only a minimal amount of ROS and recruits only mature-activated sperm cells [10]. Swim-up, on the other hand, has several disadvantages. The approach involves a distribution between several tubes of the sample to emphasize the region of contact with the culture medium in the event of samples with high volume or raised concentration; this subdivision adds to the labor. Another flaw is the minimal percentage of spermatid cells recovered; only around 10% [3] of the entire sperm cell population is suitable for further usage. Many motile cells from pellets may become attached to the bottom of the tube and never reach the growth media layer if samples are not centrifuged gently.

Some of the advantages include fact that it is Simple, quick, and cost-effective. Isolates spermatozoa that are motile and morphologically normal [11]. Non-invasive, low-ROS production and DNA that isn't fragmented [10]. The proportion of spermatozoa with chromosomal abnormalities is decreasing. [10] To improve the quality of the spermatozoa recovered after density gradient centrifugation, this procedure can be used. Some disadvantages noticed were, Reduced quantity of spermatozoa retrieved [9] In the case of big volumes, there is a lack of efficiency [5] and little ROS production.

IV. DENSITY GRADIENT METHOD (DG):

Sperm cells are centrifuged after being placed across a continuous or discontinuous density gradient in DGC [12] (Figure 1). Separation is based on density and motility, with the quickest spermatozoa migrating to the tube's bottom.

The sample is centrifuged for roughly half an hour after being placed on top of a solution with a higher density. Although all sperm cells travel through gradients, motile spermatozoa swim aggressively rather than being exposed to centrifugation kinetics. Immotile cells and detritus persist between gradients, whereas faster sperm cells form a silky pellet at the tube's bottom.

To avoid any pH or Osmolarity changes, the chemical utilized to make the gradients should not be hazardous to spermatozoa and should be stable in solution.

It is shown in previous research that hydrophilic organic molecules, such as sucrose or sucrose copolymers (Ficoll) [14], are less hazardous than mixed substances, such as colloidal silica covered with Polyvinylpyrrolidone (Percoll), and have fewer detrimental effects on ultra-sperm structure [15]. Even from a sample with low sperm motility, this approach facilitates the

isolation of motile spermatozoa. However, the morphology and motility parameters of different studies in the literature varied significantly. Different beginning conditions, such as concentration, volume, number of layers, g-force used, and centrifugation time, maybe the cause of this bias. Furthermore, the procedure is more time-consuming. When the viscosity of semen is changed, DG yields less and creates more ROS as a result of the mechanical stress induced by centrifugation. Clusters of distinct types of cells inhibit migration through gradients when samples are overly concentrated. Sadly, density gradient centrifugation is more expensive than the traditional SU [5].

Several researchers have examined morphological and dynamical factors to determine which method is the best for sperm cell production. Nonetheless, the results that have so far been published in the literature are contradictory.

V. MICROFLUIDIC METHOD:

Microfluidic is the science and technology of precisely manipulating small volumes of fluids in a microchannel of a few hundred micrometers in diameter [16]. This sub-millimeter scale has two key advantages: (I) the flows involved are laminar, allowing us to use the fluid's deterministic behavior to precisely manage the fluids and suspended particles; and (II) channel features have dimensions on the order of several biological particles (1–100 μm). Microfluidic is the term used to describe the manipulation of fluids at this scale, and microfluidic devices are the devices that use these techniques. Microfluidic devices are made up of functional microscale pieces for fluid distribution, fluid mixing [17], and particle separation in fluids, and are typically created using microfabrication techniques [18]. In addition, many types of microfluidic systems can be combined to create highly sophisticated systems with small footprints. Microfluidic devices are also known as Micro-Total-Analysis Systems (TAS) or lab-on-a-chip (LOC). Microfluidic devices are used in analytical chemistry, molecular and cellular biology, microbiology, and pharmaceutical drug screening at the moment [19]. Microfluidic devices have been created to supplement or perhaps replace conventional sample manipulation techniques such as cell culture, cell separation, and DNA analysis in all of these domains.

The capacity to evaluate essential semen analytic characteristics such as sperm count, sperm motility, and sperm morphology has been demonstrated by several microfluidic devices. These technologies have regularly proven to be fast, simple to use, and accurate. Microfluidics also has the potential to meet the demand for fertility diagnostics in point-of-care settings and poor countries [23].

Severing and co-workers [24] created a microfluidic device that used the electrical impedance approach, also known as the Coulter Principle—an established method for cell counting and size analysis—to precisely estimate sperm count. All semen samples in the subfertility zone (20106 m/L) were correctly categorized by this method, which had a significant association with traditional methods.

Microfluidic on paper has recently developed as a simple, low-cost, and speedier alternative to traditional sperm analysis. They have the potential to offer effective at-home semen analysis, similar to pregnancy testing equipment. The paper-based microfluidic device developed by Nonrated and colleagues for at-home semen testing is one promising example [25]. Microfluidic systems demonstrate various advantages over conventional bulk fluid techniques by utilizing multiple aspects highlighted that are specific to the microscale.

1. Microfluidic systems can work with samples of very small volumes (mL to nil). As a result, the operational cost is lowered as the volume of expensive reagents is reduced [19].
2. Microfluidic systems are sensitive and have quick response times. At the microscale, increasing the surface-to-volume ratio, diffusion rate, and heat dissipation rate enhances detection limits and sensitivity while also reducing reaction times [19].
3. Large-scale parallelization for high throughput operation and numerous parameter experiments is possible with microfluidic systems. During the manufacturing of microfluidic devices, multiple functional structures can be simply parallelized. For example, high throughput and high-resolution analysis for drug screening and antigen detection has been obtained by embedding a microplate system array in a microfluidic chip [20];
4. Single-cell analysis can be done with microfluidic technologies. The micro scale's functional structure and forces allow for accurate single-cell manipulation and the separation of rare cells in highly heterogeneous mixtures. In microfluidic devices, physical trapping, immunological capture, and optical tweezers can be used to manipulate cells [18].
5. Microfluidic systems can perform sample treatment and analysis automatically. Microfluidic systems are a great way to automate reagent delivery and condition monitoring [21]. Furthermore, portable point-of-care diagnostic tools based on microfluidic systems enabling on-site quick sample analysis can be built [22].

VI. Magnetic Cell Activation Method

Apoptotic sperm with significant quantities of fragmented DNA can be separated from the rest using magnetic-activated cell sorting (MACS), enhancing the overall quality of the seminal sample. Apoptosis is a physiological mechanism that removes defective spermatozoa from the testes in order to maintain testicular homeostasis in terms of germ-cell population and testicular nutrition availability [26]. Nuclear endonucleases are activated during apoptosis, resulting in the formation of double-strand breaks (DSBs) on sperm DNA. These are linked to miscarriage and implantation failures [27].

Sertoli cells can identify sperm cells containing apoptotic signals on the outer plasma membrane, such as phosphatidylserine (PS) residues, and remove them. Sertoli cell dysfunction or over-activity of testicular apoptotic processes (known as abortive apoptosis) might hamper apoptotic cell clearance during spermatogenesis, resulting in an increase in the number of apoptotic sperm cells in the ejaculate [29].

Phagocytes can recognize and efficiently eliminate apoptotic sperm cells with externalized PS residues on the plasma membrane in the female genital tract under normal physiological conditions [30], preventing the fertilization of the oocyte by a spermatozoon with alterations in its DNA integrity. When ART sperm-selection techniques like density-gradient centrifugation or sperm swim-up are used, this critical sperm-selection step is inconveniently ignored. Because these procedures rely solely on the recovery of sperm populations with a high motile capacity, this is the case. Furthermore, in intracytoplasmic sperm injection (ICSI) cycles, the embryologist's subjective choice for the best sperm morphology is used to select the viable spermatozoon to be injected into an oocyte. As a result, the physiological identification of non-functional and viable sperm in the female genital canal is not included in these processes.

Different sperm-selection approaches have recently been developed to address this issue, based on various functional features and activation mechanisms that occur during sperm-cell Capacitation. One is the magnetic-activated cell-sorting (MACS) technology, which is utilized to identify and positively eliminate apoptotic cells from ejaculate [31] using Annexin V-conjugated superparamagnetic microbeads to recognize externalized PS residues on apoptotic sperm cells [32]. Before using the ejaculate for ART operations, this approach lowers the amount of sperm with fragmented DNA [33]. Furthermore, in newborns that were extensively observed following oocyte donation and sperm selection treatments with MACS, there were no clinically harmful consequences at the obstetric and perinatal levels.

The benefits of the MACS approach in ART continue to be a source of debate. The MACS selection strategy has been verified in certain articles as a useful method for reducing the amount of apoptotic sperm, hence enhancing overall embryo quality and conception rates [34].

VII. CONCLUSION:

The effectiveness of ARTs is a significant factor to consider while treating infertility. In ARTs, sperm selection is critical for achieving greater live birth rates, particularly in cases when the male factor is present. This article discusses the different methods of SU, DGC, Microfluidic & MACS methods of sperm preparation, presenting various aspects of each of them. Even though the current methods are developing, there is a lot of scope for the future to improve and fine-tune the processes existing.

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