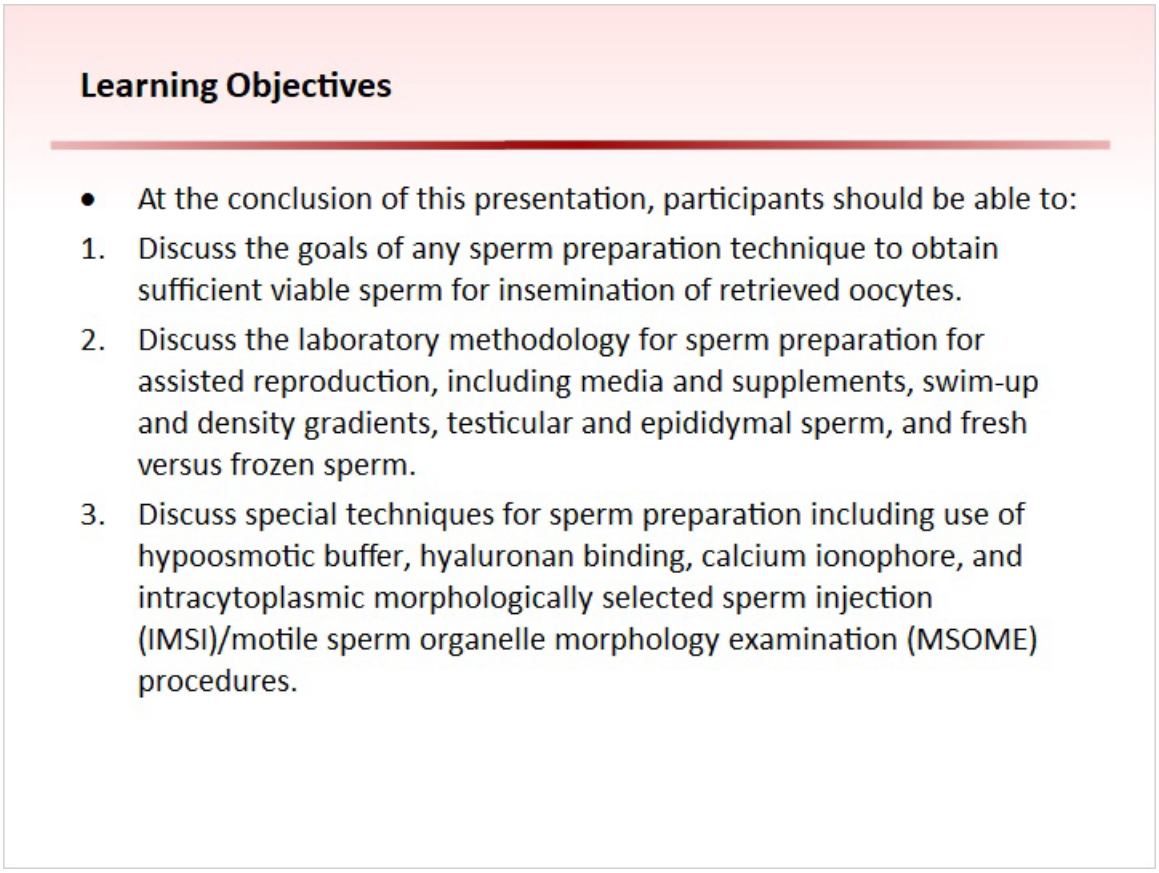
# LABCC100 Lesson 17

## *1.1 Preparation and Selection of Sperm for IVF and ICSI*


**Notes:**

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Preparation and Selection of Sperm for IVF and ICSI.

## *1.2 Learning Objectives*



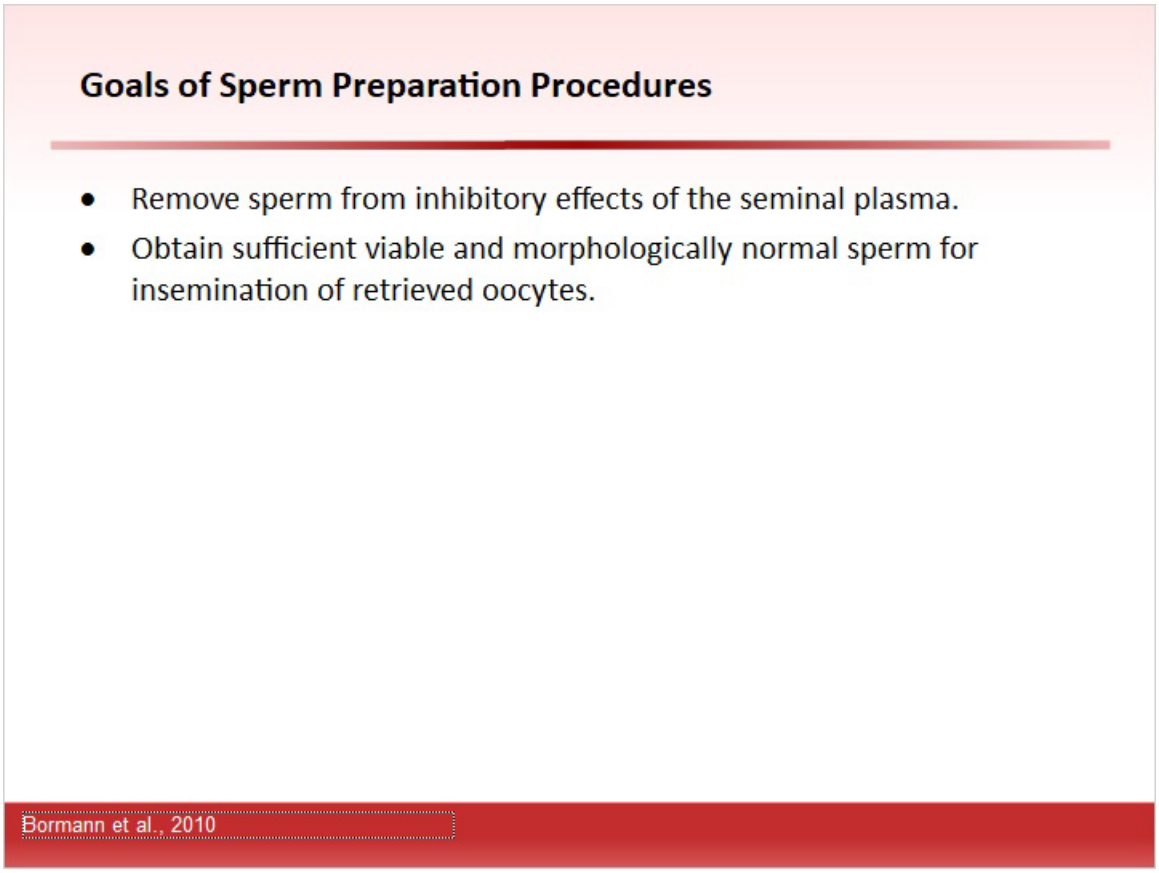

**Notes:**

At the conclusion of this presentation, participants should be able to:

- Discuss the goals of any sperm preparation technique to obtain sufficient viable sperm for insemination of retrieved oocytes.
- Discuss the laboratory methodology for sperm preparation for assisted reproduction, including media and supplements, swim-up and density gradients, testicular and epididymal sperm, and fresh versus frozen sperm.

Discuss special techniques for sperm preparation including use of hypoosmotic buffer, hyaluronan binding, calcium ionophore, and intracytoplasmic morphologically selected sperm injection (IMSI)/motile sperm organelle morphology examination (MSOME) procedures.

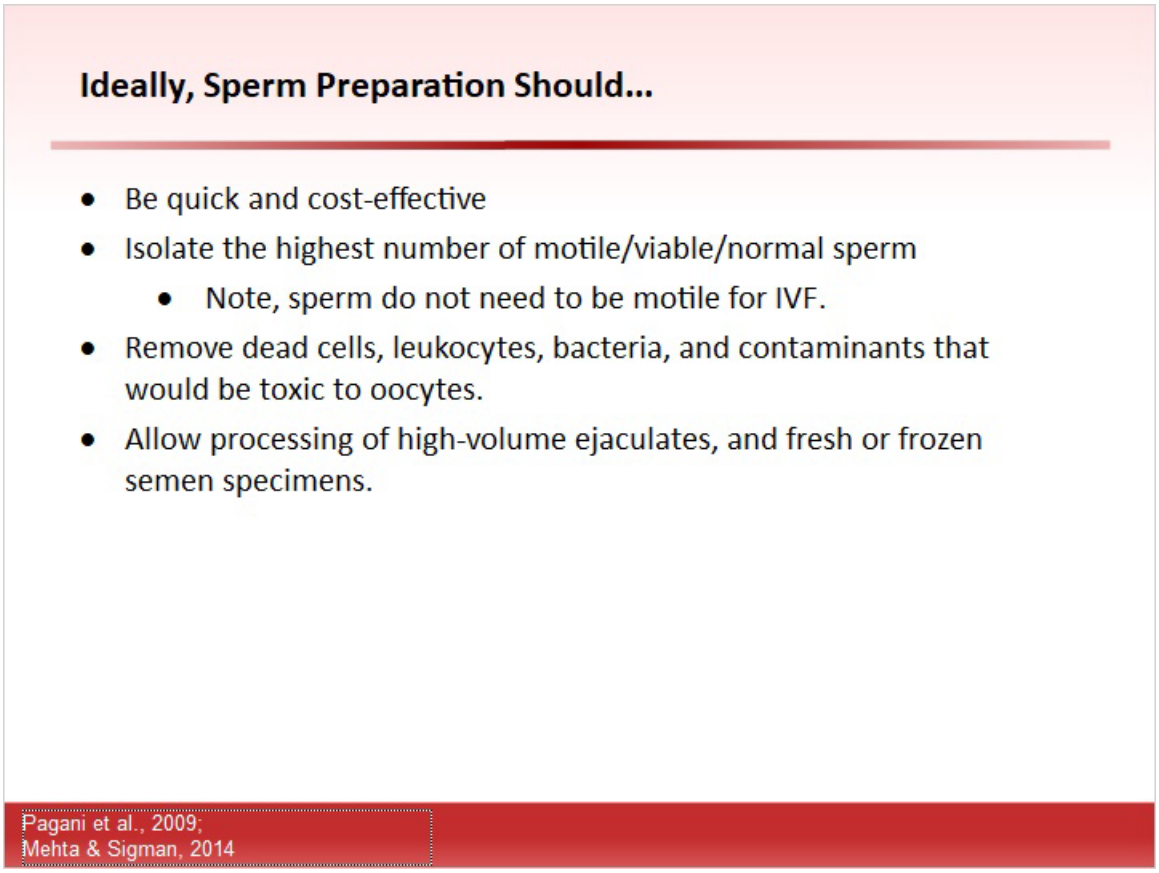
## *1.3 Goals of Sperm Preparation Procedures*




**Notes:**

The goal of any sperm preparation method is first to remove sperm from inhibitory factors present in the seminal plasma. Factors in seminal plasma inhibit spermatozoa from undergoing capacitation and the acrosome reaction and reduce the ability to successfully fertilize an oocyte. In addition, it is obvious that the ultimate goal of any sperm preparation method is to obtain sufficient viable and morphologically normal sperm for use in the selected assisted reproductive technology (ART).

## *1.4 Ideally, Sperm Preparation Should...*

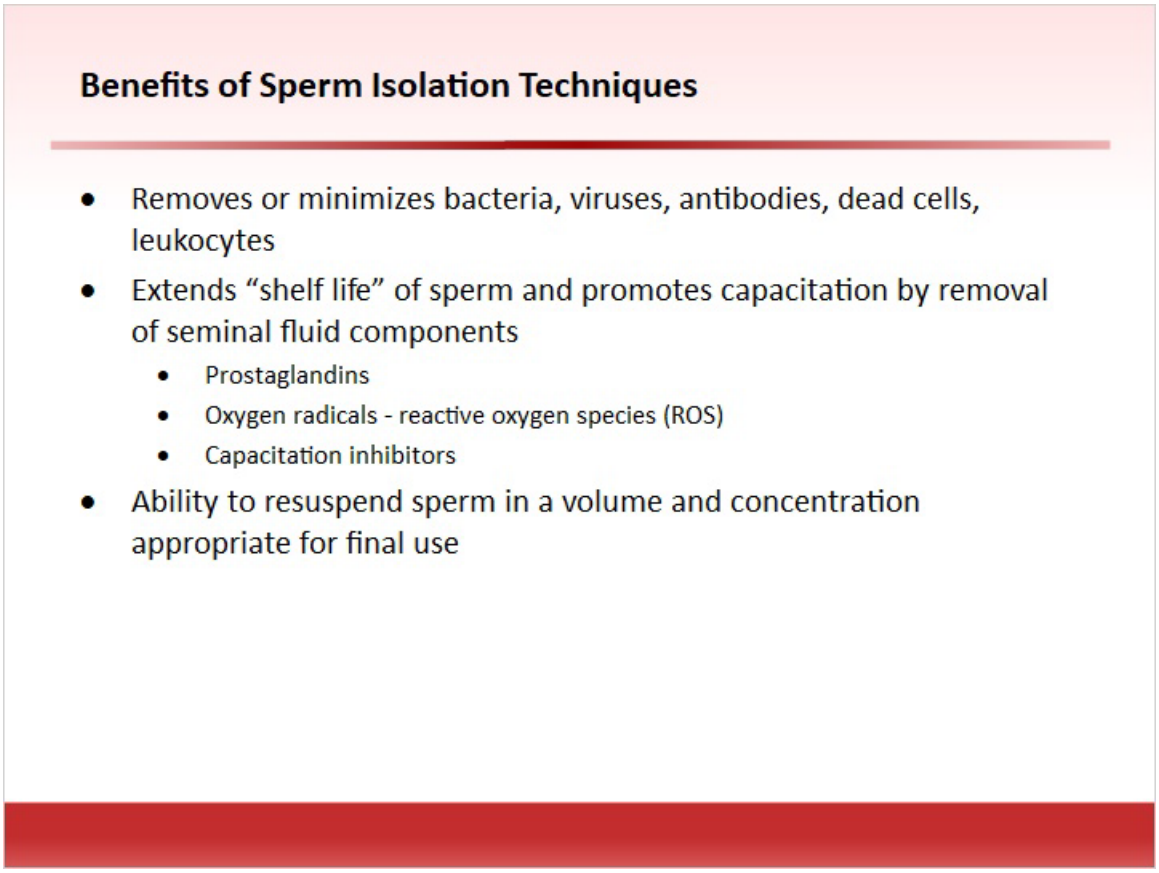



**Notes:**

Ideally, sperm preparation should be quick, easy, and nontoxic, isolating the highest concentration of motile, viable, and morphologically normal sperm. Procedures should also remove dead cells, leukocytes, bacteria, and toxic or bioactive substances-all of which would be harmful to oocytes. Finally, the process should allow processing of high-volume ejaculates as well as fresh or frozen specimens and tissue biopsies, such as testicular biopsy tissue or epididymal aspirates. No one technique exhibits all of these requirements.

Note that sperm do not need to be motile to be used for in vitro fertilization (IVF), or intracytoplasmic sperm injection (ICSI). The sperm must be viable and morphologically normal. The technique of choice will depend on both the quality of the sperm specimen and the end use of the sperm for IVF, ICSI, or intrauterine insemination (IUI).

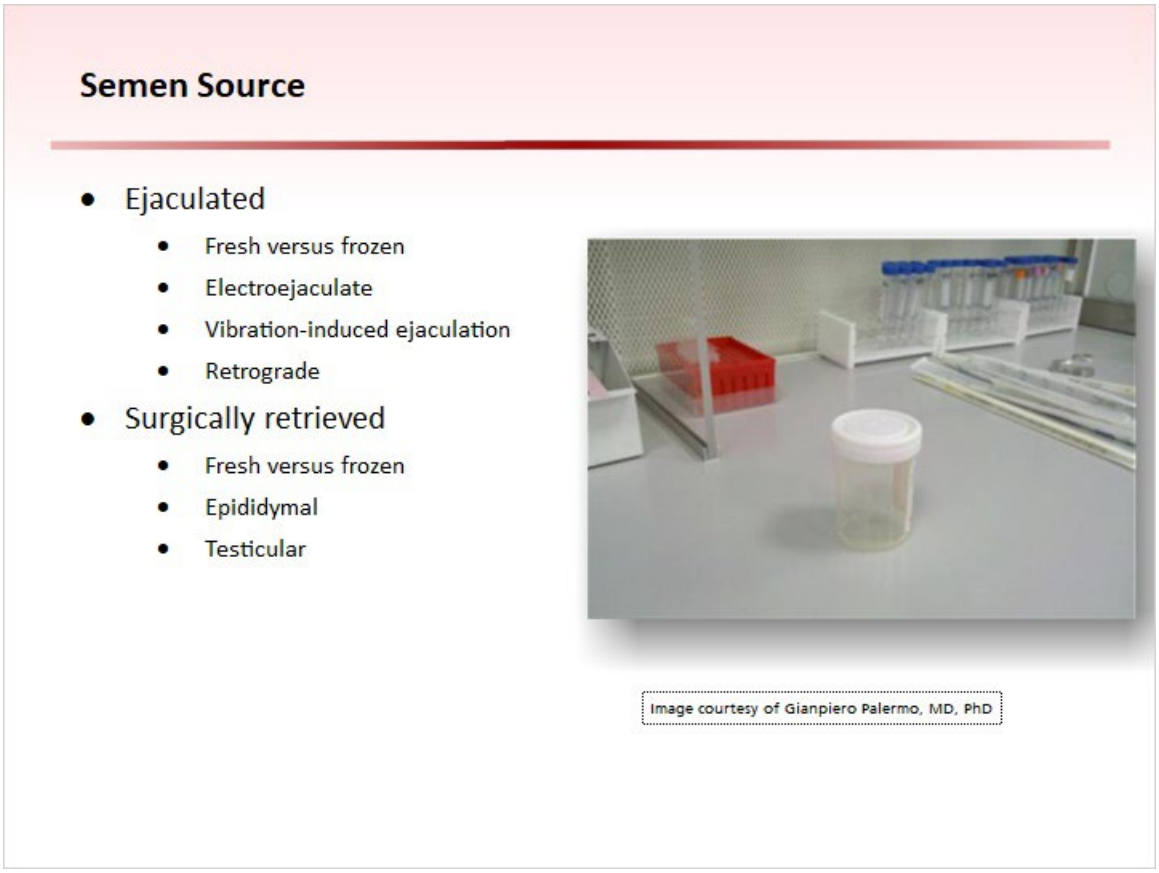
## *1.5 Benefits of Sperm Isolation Techniques*




**Notes:**

In summary, sperm isolation procedures should remove or minimize viruses, bacteria, dead cells, and leukocytes from the sperm, since exposure can be harmful to the sperm and to the oocytes. In doing so, such a procedure will extend the life of sperm, and promote capacitation and the acrosome reaction. Finally, any sperm preparation procedure should result in a final volume of sperm fraction that is appropriate for the final use of the sperm: IVF, ICSI, IUI.

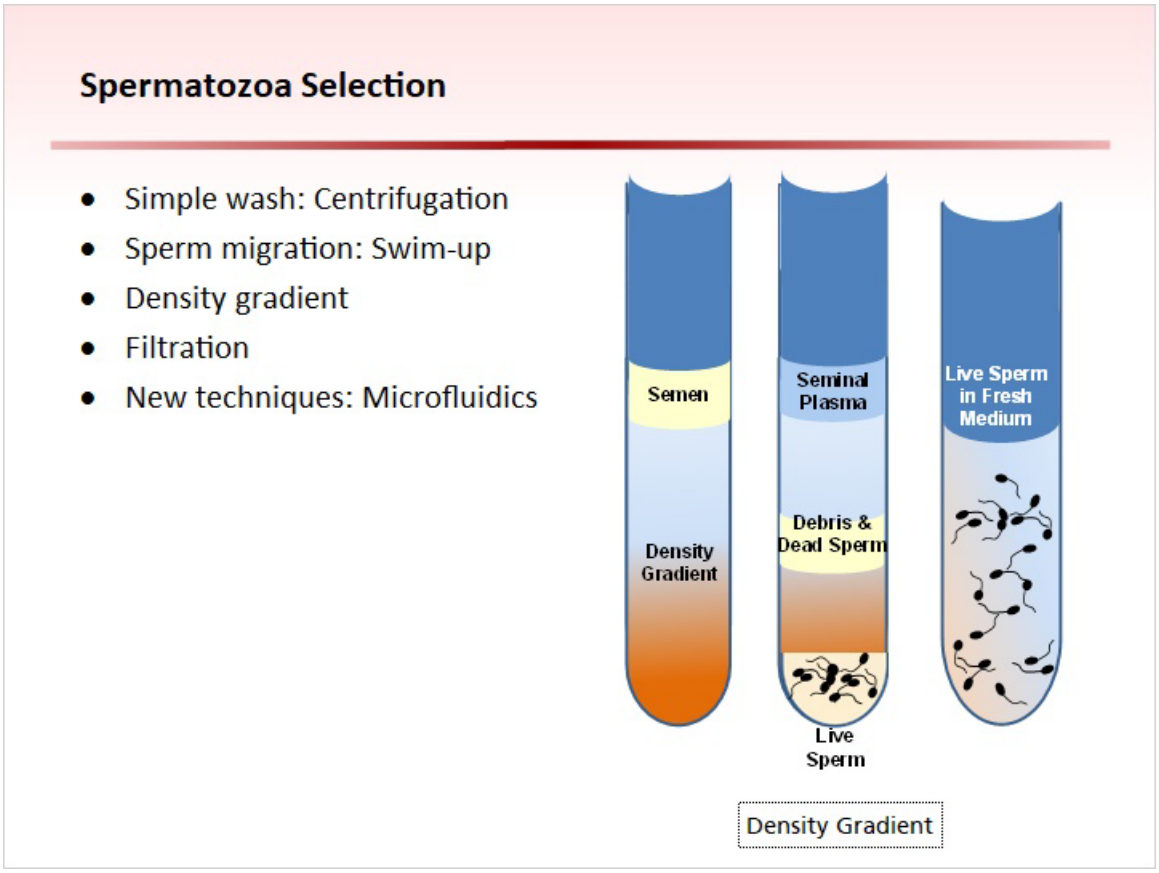
## *1.6 Semen Source*




**Notes:**

Although sperm are typically thought of as originating from ejaculated semen, many different sources exist. Sperm can either be ejaculated or surgically retrieved. Ejaculated sperm include fresh semen, frozen-thawed semen, electroejaculated semen, and retrograde semen, which is semen recovered from the urine. Special preparations are necessary to obtain electroejaculated semen from spinal cord-injured men, as well as semen from the bladder in men with retrograde ejaculation (due to diabetes, result of pelvic surgery, etc.). Surgically retrieved sperm originate from epididymal aspiration or testicular biopsy.

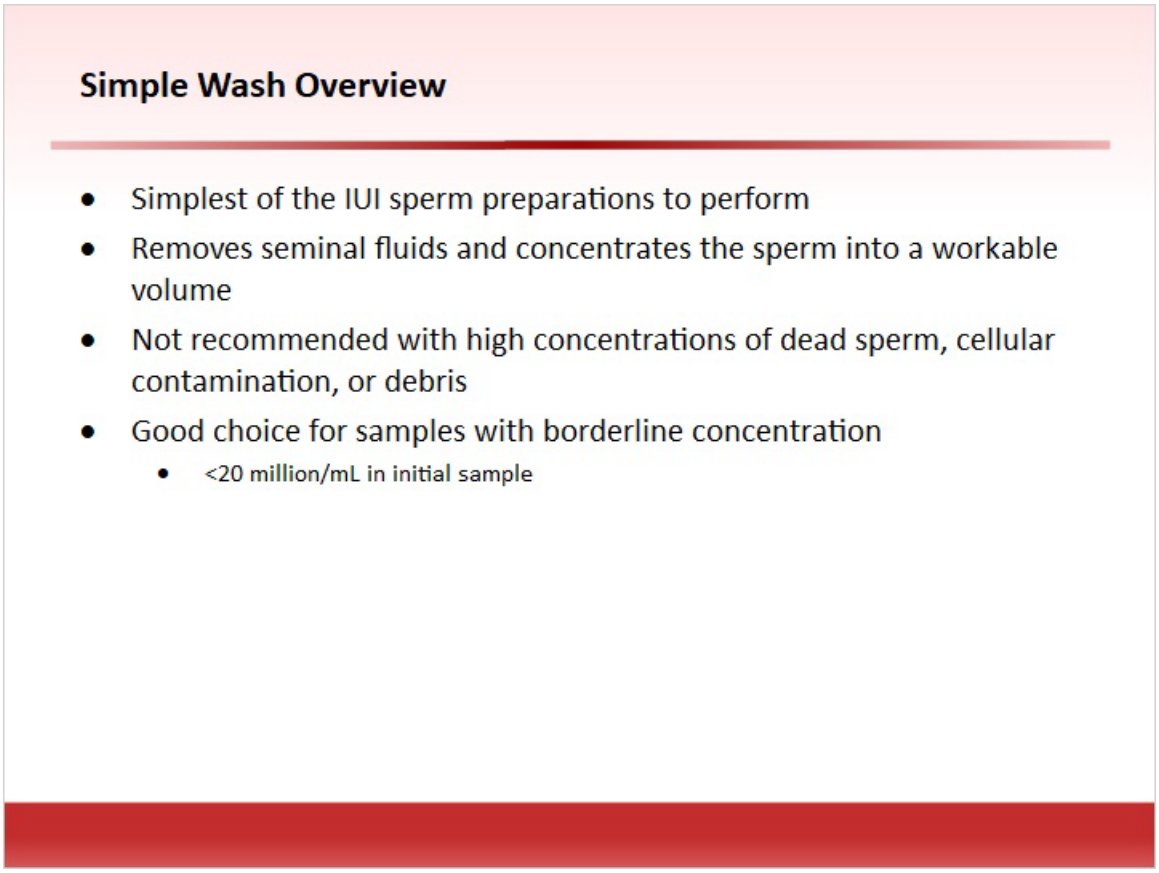
## *1.7 Spermatozoa Selection*




**Notes:**

Raw semen must undergo processing in order to best prepare the sperm for fertilization. Several techniques have been developed to isolate sperm for use in assisted reproduction. These include 1) simple wash-centrifugation; 2) migration techniques including swim-up from a washed pellet or directly from semen; 3) density-gradient centrifugation; 4) filtration through glass-wool or dextran gel beads; and 5) new techniques including microfluidics. Each of these procedures will be reviewed briefly.

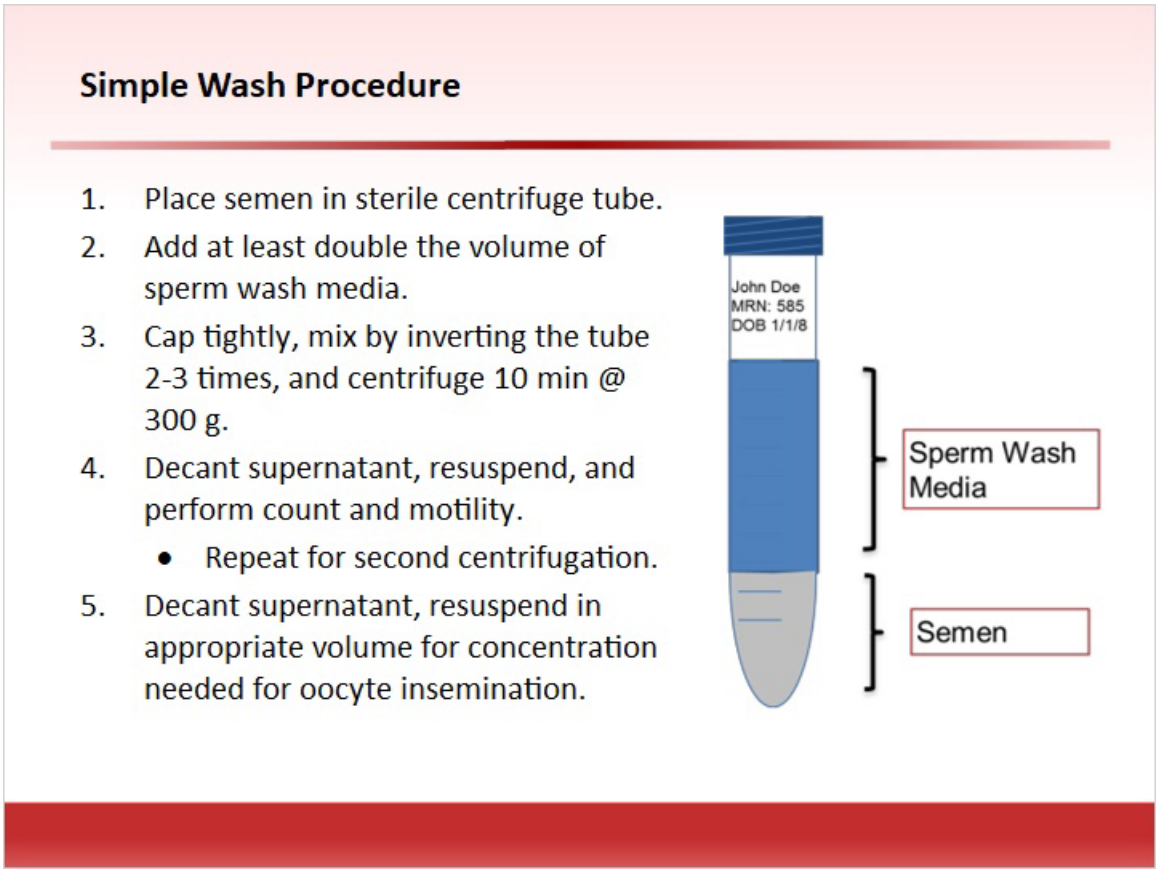
## *1.8 Simple Wash Overview*




**Notes:**

The simplest technique for preparing sperm is a simple wash procedure, where the fresh semen, diluted with nutrient media is centrifuged, usually twice, and the supernatant removed. The final pellet of sperm is resuspended in a volume appropriate for the end use of the sperm. A simple wash procedure is not recommended for specimens with high concentrations of dead sperm or debris, since dead sperm, cells, and debris would not be removed, and would be pelleted with the live sperm. This technique is advantageous for specimens clean of debris and cells, and those with lower concentrations. A single wash-centrifugation method is commonly used for frozen-thawed sperm.

## *1.9 Simple Wash Procedure*




**Notes:**

A semen analysis should be conducted on each sample before and after the preparation. If a sample is highly viscous, it may be necessary to either give the sample more time to liquefy, draw it back and forth through a blunt needle and syringe or small pipette to help break up the sample, or wash it a second time if the sperm do not pellet well at the bottom of the tube after an initial wash. Generally speaking, it is best to minimize manipulation of the sperm, such as with extra washes, etc. The basic wash procedure is to place the semen in a sterile centrifuge tube and add at least double the volume of sperm wash media. After capping tightly, mix by inverting the tube 2-3 times then centrifuge 10 minutes at 300 g. This step is generally repeated for a second centrifugation/wash. Finally, decant the supernatant, and resuspend pellet in 0.6-0.7 mL fresh media for insemination. Once a preparation is completed, the final sample should be drawn into a syringe or packaged in some way for use, such as for IUI, or placed into a sterile tube and provided to the IVF lab.

## *1.10 Simple Wash*

### Simple Wash

**Advantages**

- Simple and quick to perform
- High recovery of sperm
- Requires minimal processing of sperm

Image courtesy of Gianpiero Palermo, MD, PhD

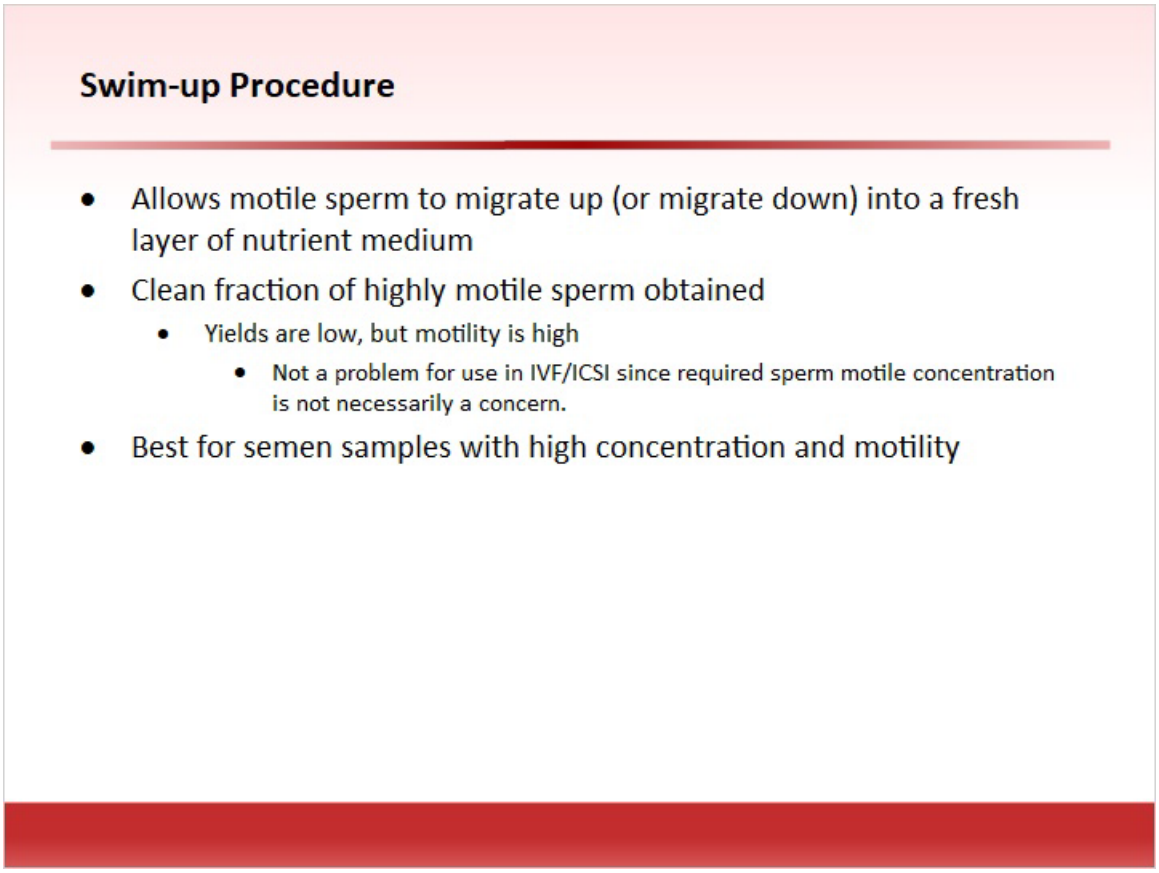
**Disadvantages**

- Motile sperm concentrated in presence of dead sperm, white blood cells, and other seminal debris, which expose sperm to higher levels of reactive oxygen species that may negatively affect sperm function
- Usually not recommended alone for IVF (standard insemination)

**Notes:**

Advantages and disadvantages of the basic wash procedure are listed here. Advantages include that it is easy and quick to perform, uses sterile plasticware (centrifuge tubes, pipettes, media), and takes little time (30 minutes including all steps). This procedure also results in a high recovery of sperm and requires minimal processing steps. However, with a simple wash, motile sperm are concentrated in the presence of non-motile and dead sperm, white blood cells, and other seminal debris. This exposes sperm to higher levels of reactive oxygen species that may negatively affect sperm function.

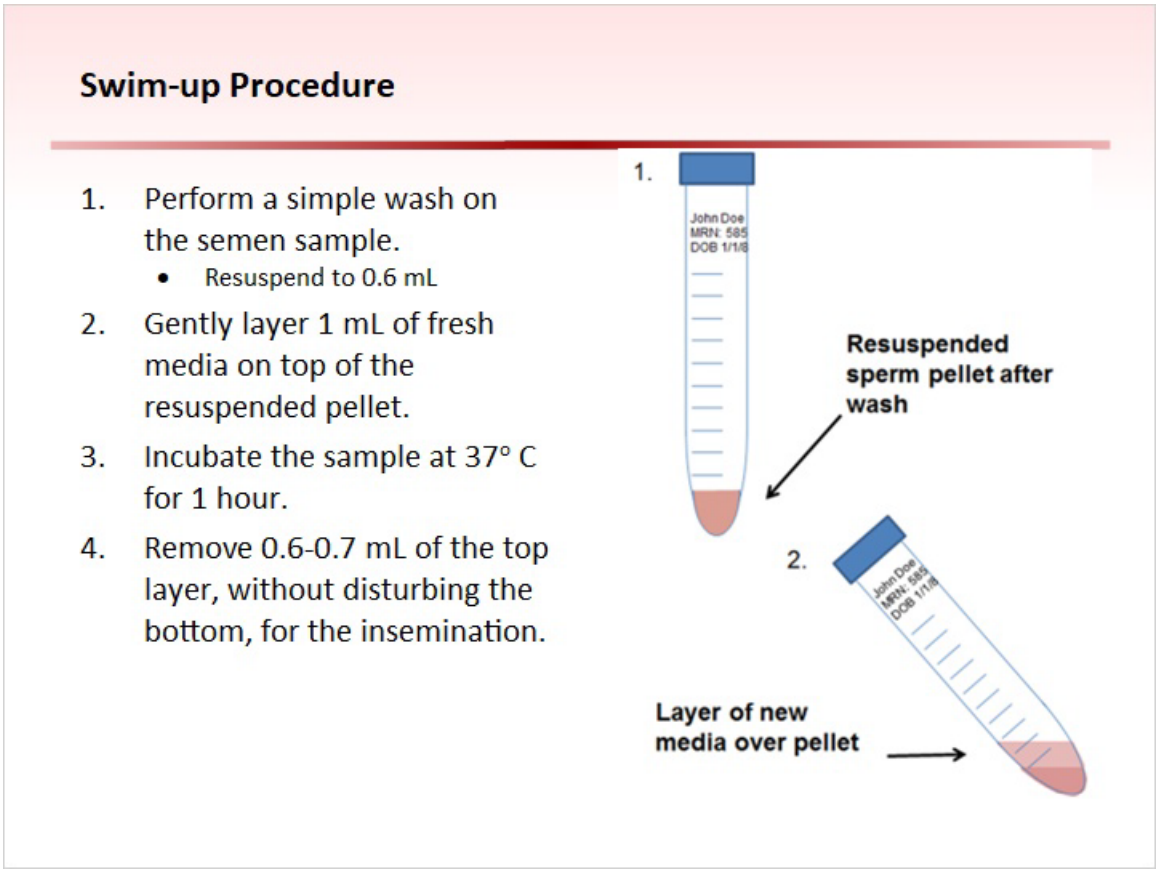
## *1.11 Swim-up Procedure*




**Notes:**

A second, commonly used sperm preparation procedure is a swim-up. This procedure allows sperm to migrate, or swim up into a fresh layer of nutrient medium. Motile sperm are a prerequisite since motile sperm can only migrate into the media. With swim-up, a clean fraction of highly motile sperm can be obtained, but the concentration of motile sperm is low. The swim-up is best for specimens with high concentration and motility. With IVF/ICSI procedures, low yield is not usually a concern since fewer sperm are needed for oocyte insemination.

## *1.12 Swim-up Procedure*




**Notes:**

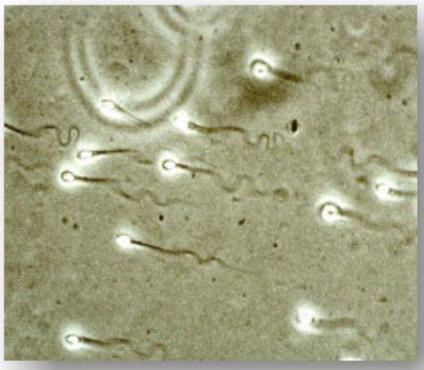
There are several variations of how a swim-up can be performed. The semen can be diluted with nutrient media, centrifuged into a pellet. Fresh media can be layered over the washed pellet or a resuspended sample. The top layer can be removed without disturbing the bottom and further concentrated or diluted depending on the insemination (IUI or IVF/ISCSI). The protocol shown here uses a resuspended sample rather than a pellet to reduce the exposure of healthy sperm to reactive oxygen species that could negatively affect sperm function. It is also helpful to incubate the swim-up at an angle, if possible, to increase the surface area between the layers and improve motile sperm recovery.

## *1.13 Swim-up from Washed Sperm Pellet*

### Swim-up from Washed Sperm Pellet

**Advantages**

- Cost-effective.
- Very "clean," highly motile final sample



**Disadvantages**

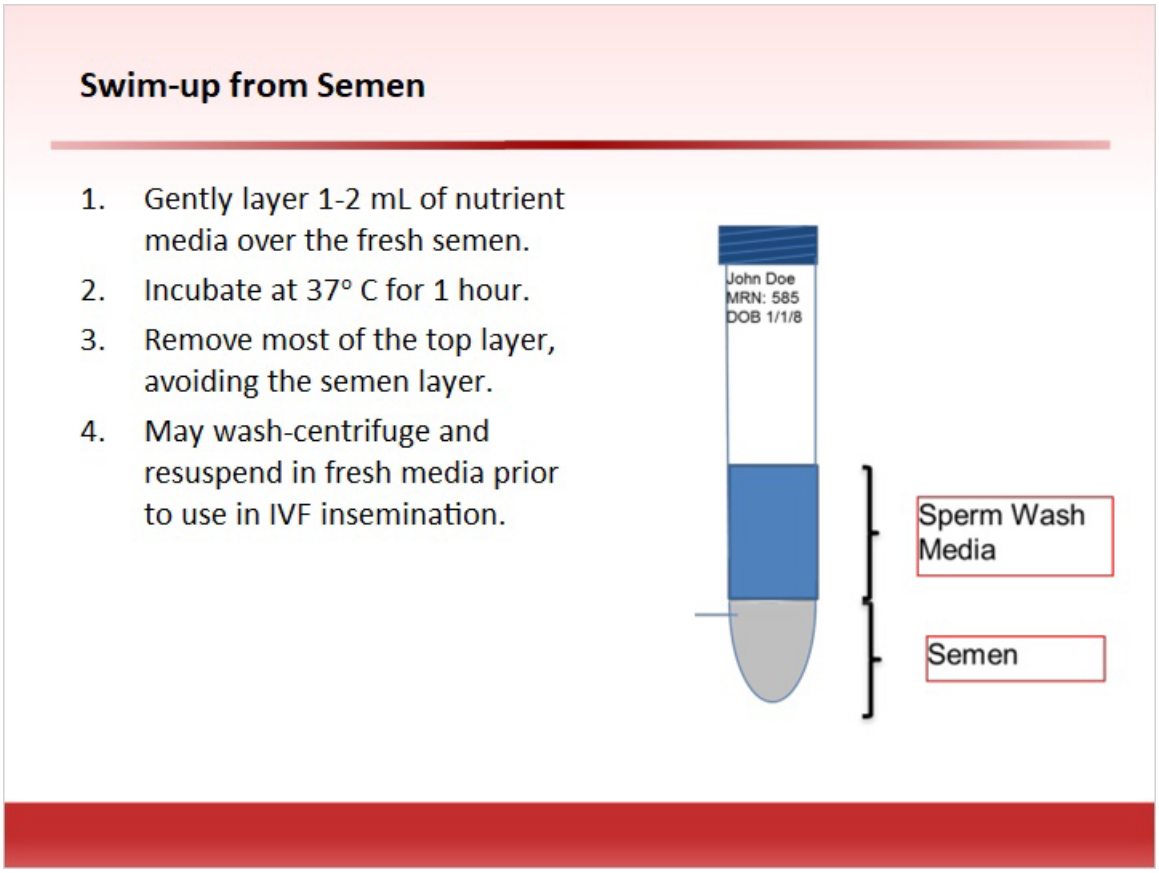
- Centrifugation of semen into a pellet increases exposure of motile healthy sperm to dead cells and debris, ROS.
- Not advantageous for specimens with reduced motility and/or concentration, or non-motile sperm
- Takes longer time to perform

ROS = reactive oxygen species

**Notes:**

The swim-up method is cost-effective and provides for a very "clean," highly motile final sample. It does, however, take longer to perform than other preparations and the final concentrations may be low. A disadvantage of the swim-up from pellet technique is once again the problems with centrifuging raw semen into a pellet: motile, viable sperm are centrifuged with dead sperm, cells and debris, increasing the exposure of the "good" sperm to oxygen radicals (ROS). The swim-up method also takes longer to perform, and is not of benefit to specimens with reduced motility and/or concentration.

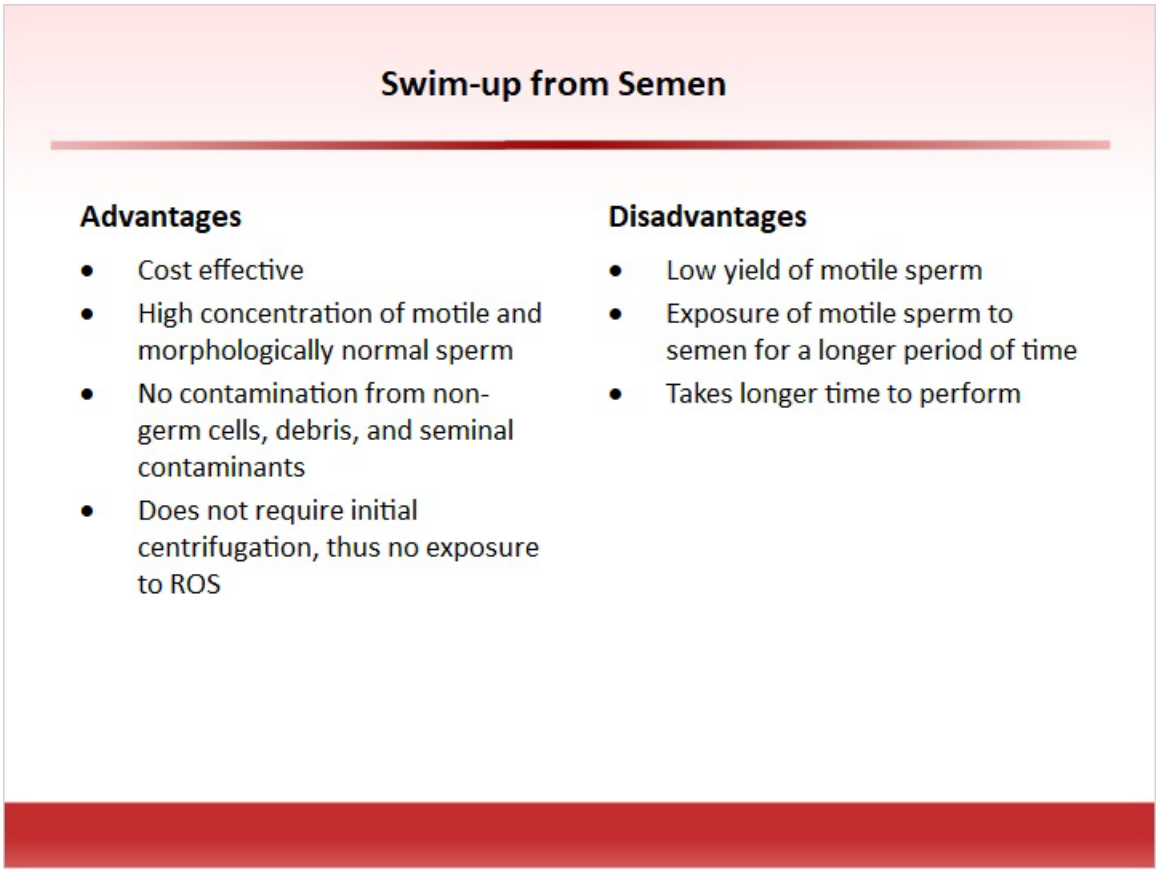
## *1.14 Swim-up from Semen*




**Notes:**

With swim-up from semen, there is no initial centrifugation of the semen into a pellet. Fresh media is layered onto the semen and incubated for approximately 1 hour to allow motile sperm to migrate into the nutrient media. The top layer of media, presumably containing the motile sperm, is removed, and may be washed again to remove remnants of seminal fluid, resuspending the sperm fraction in fresh media.

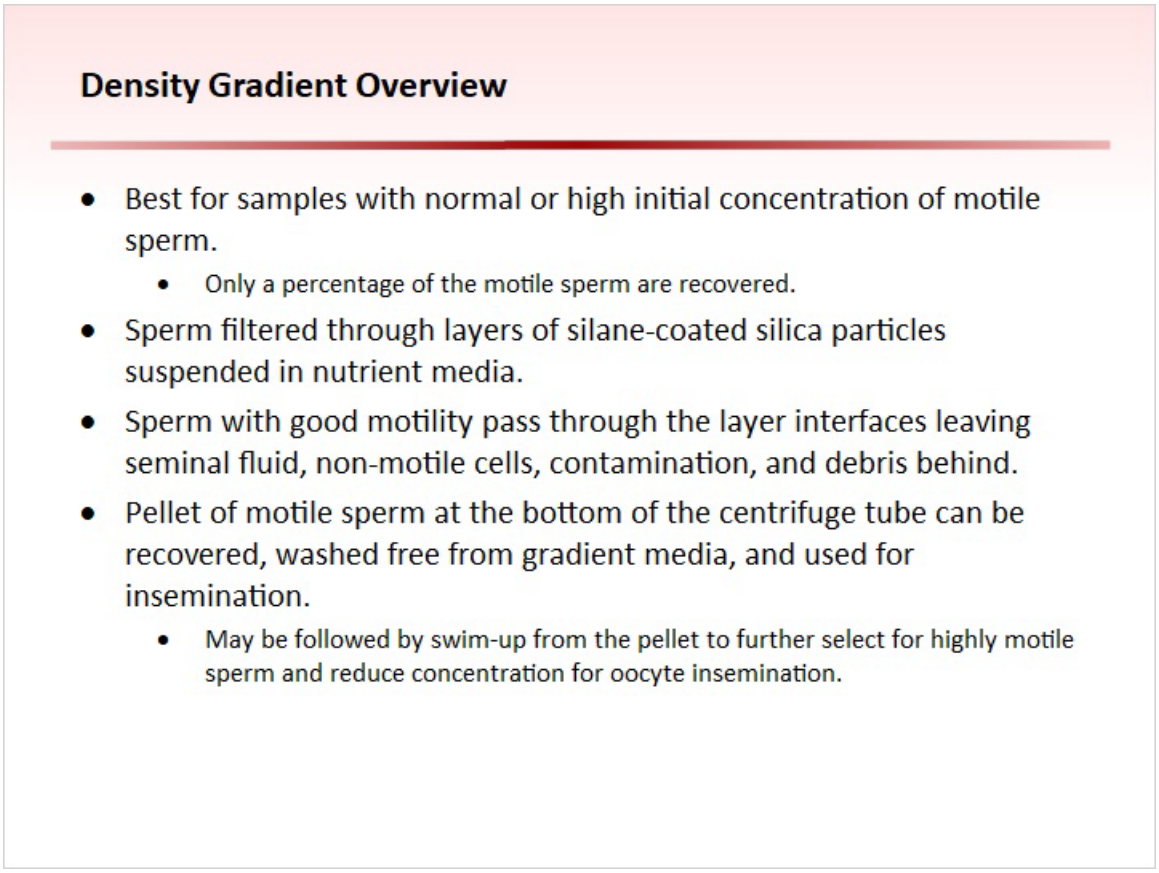
## *1.15 Swim-up from Semen*




**Notes:**

The swim-up method is also cost-effective and provides for a very "clean," highly motile final sample. It does take longer to perform than other preparations and the final concentrations may be low, although this might not necessarily be a concern for IVF/ICSI. Most importantly, this method does not require initial centrifugation of the semen, and thus avoids exposure of viable sperm to ROS from dead cells and leukocytes. It is important to set up the tubes, and begin the incubation as soon as possible after semen liquefaction to reduce exposure of the sperm to the seminal fluid.

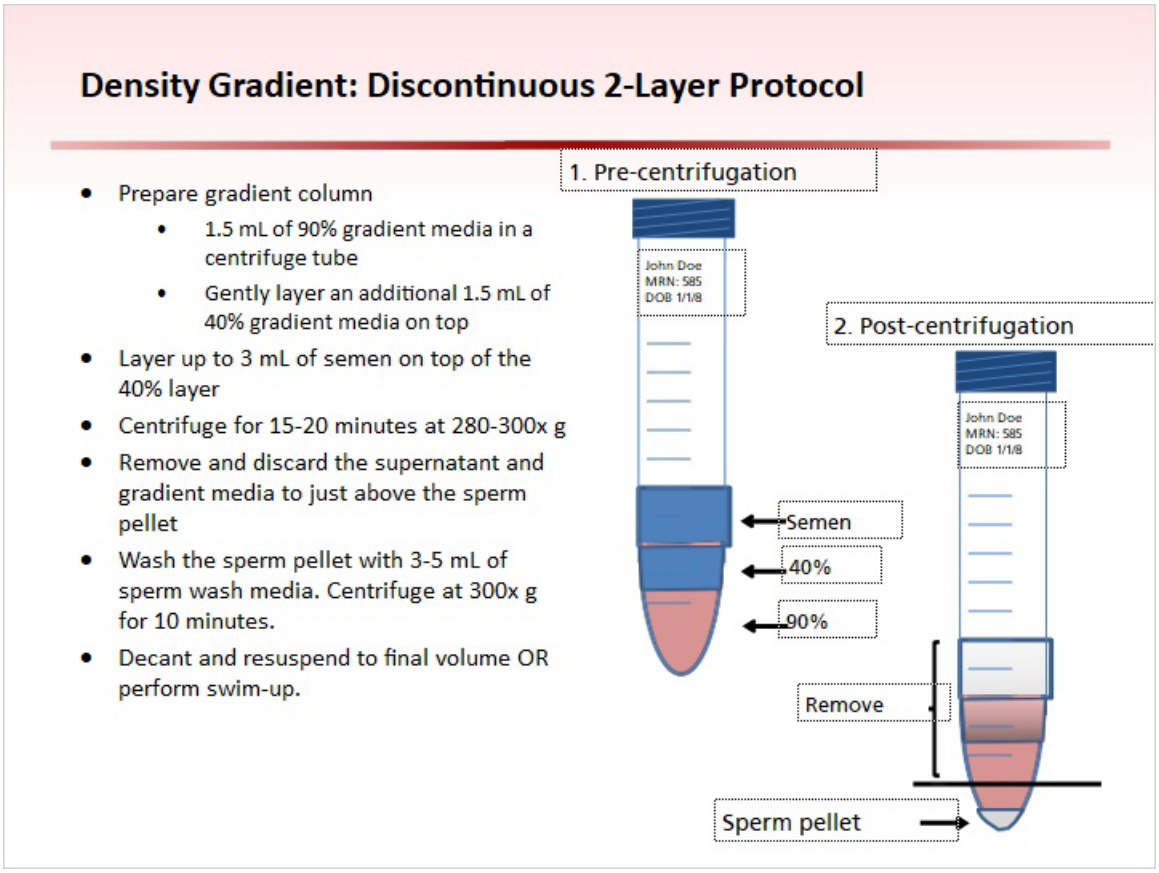
## *1.16 Density Gradient Overview*




**Notes:**

The density-gradient preparation works best for samples with a normal or high initial concentration of motile sperm, because only a percentage of the motile sperm are recovered. It should be recommended in all cases where white blood cells in the semen sample exceed the normal baseline. In density-gradient preparations, sperm are filtered through layers of silane-coated silica particles suspended in nutrient media. Sperm with good motility pass through the layer interfaces leaving seminal fluids, non-motile cells, contamination, and debris in the interface between the gradient layers and semen. The pellet of motile sperm at the bottom of the centrifuge tube can be recovered, washed free from the gradient media, and used for insemination. For IVF, density-gradient centrifugation can be followed by a swim-up to further select for a highly motile and clean preparation.

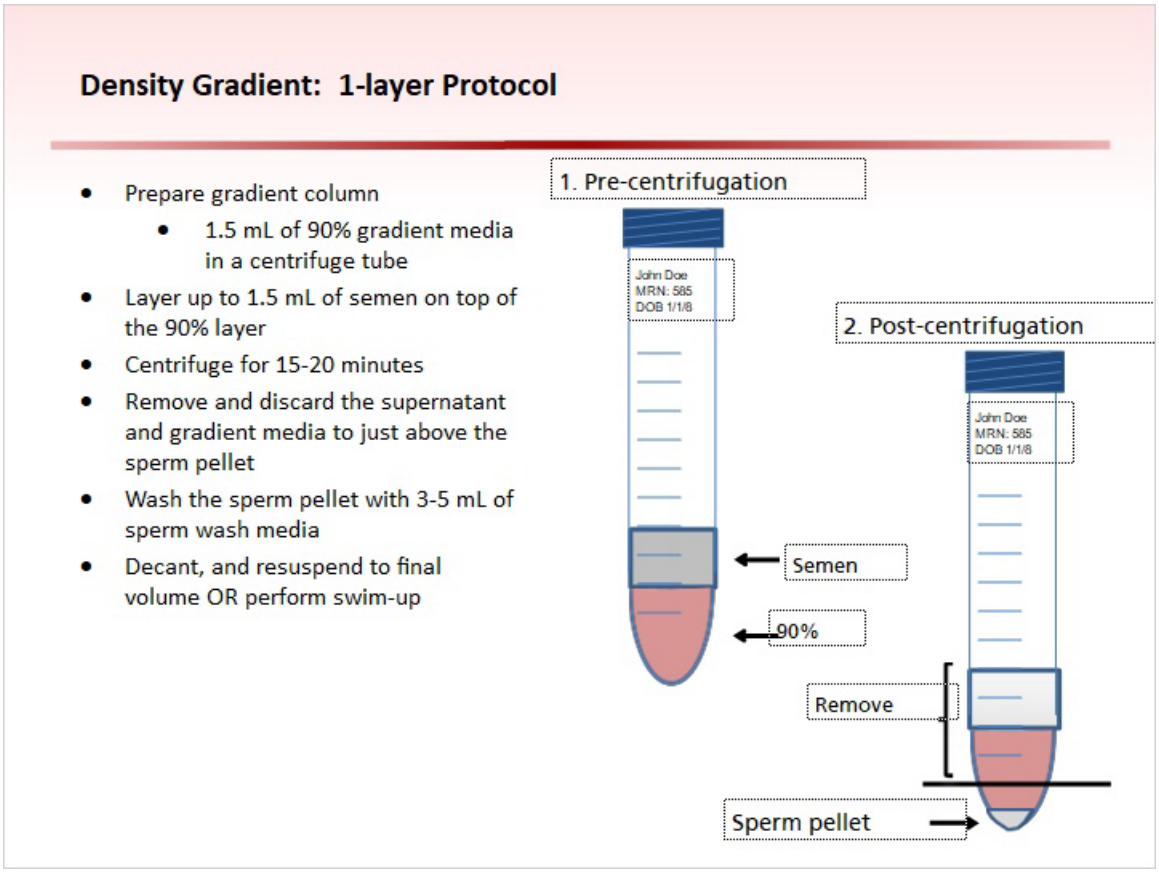
## *1.17 Density Gradient: Discontinuous 2-Layer Protocol*




**Notes:**

The density gradient preparation can be performed with several variations. First, prepare a gradient column by placing 1.5 mL of 90% gradient media in a centrifuge tube and gently layer an additional 1.5 mL of 40% gradient media on top of the 90% media. Next, layer up to 3 mL of semen on top of the 40% layer and centrifuge for 15 minutes at 300 x g. Each centrifuge has to be set for speed based on revolutions per minute, to equal the g force (300 x g) that is needed. Remove and discard the supernatant and gradient media to just above the sperm pellet. Wash the sperm pellet with 3-5 mL of sperm wash media, then decant, and resuspend to final volume. The concentration of the gradient media layers can be altered, or a single layer of concentrated gradient media can be used, if preferred. As a general rule, the total volume of semen processed should not exceed the volume of the media in the gradient column. Recovery of motile sperm can also be decreased if the gradient layers are too large. If it is desired to process more semen, extra tubes with gradient columns can be prepared and used.

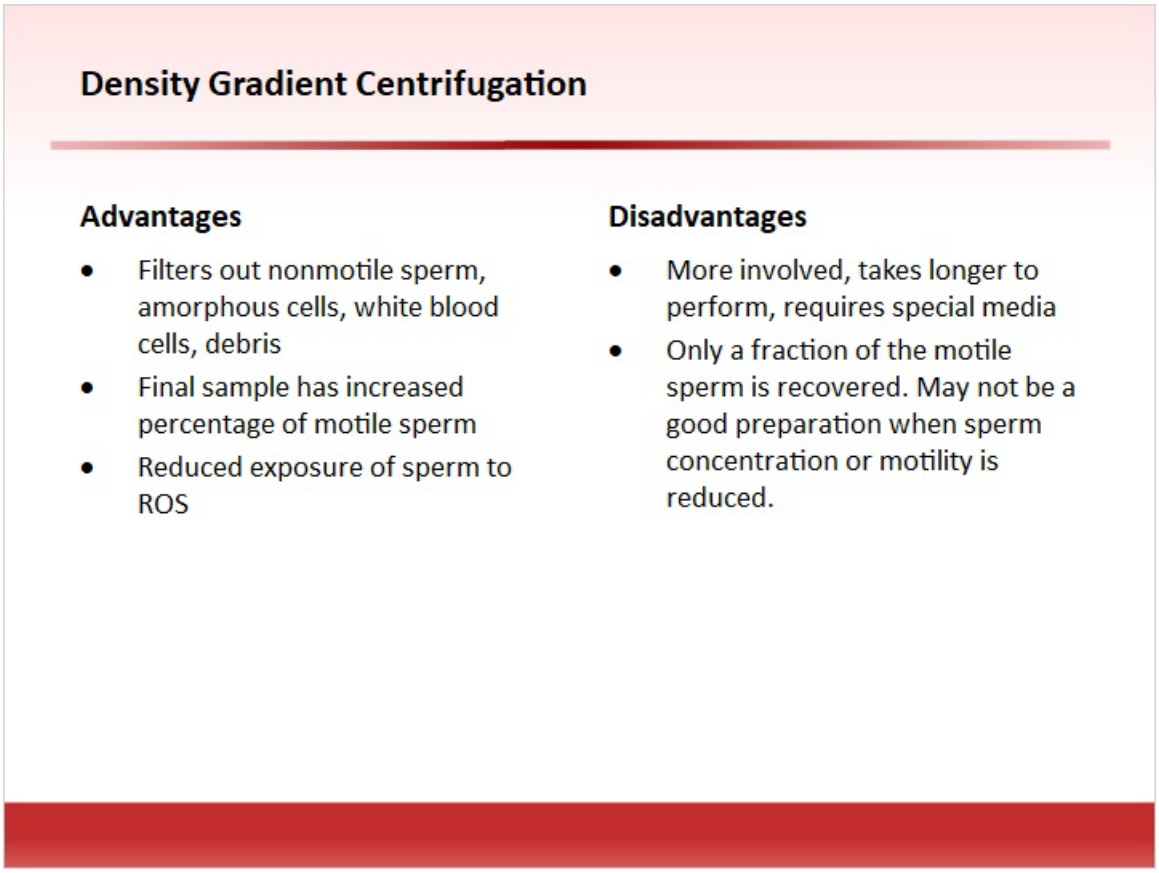
## *1.18 Density Gradient: 1-layer Protocol*




**Notes:**

The density-gradient preparation can be performed using one layer of gradient solution, usually 80% or 90%. The procedure is the same for all subsequent steps.

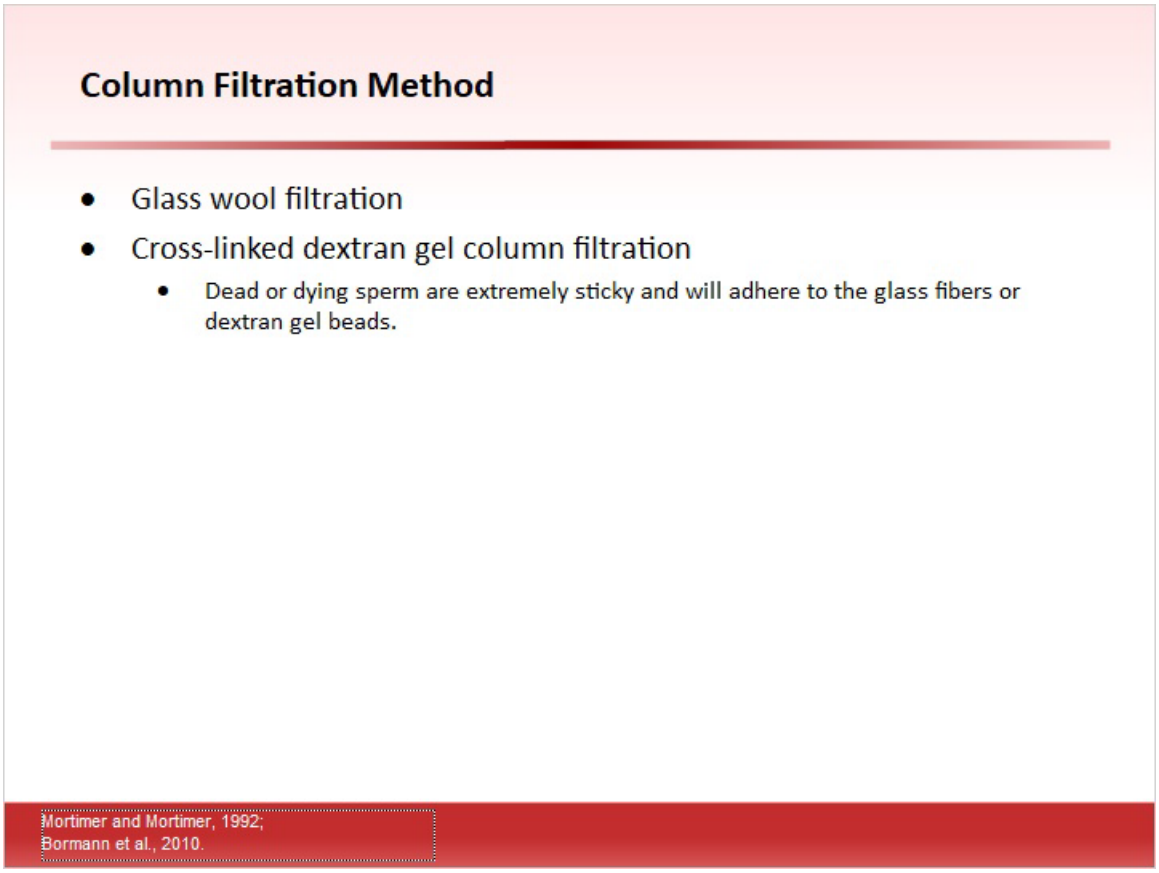
## *1.19 Density Gradient Centrifugation*




**Notes:**

The density gradient preparation filters out nonmotile sperm, amorphous cells, white blood cells, and debris; the final sample has an increased percentage of motile sperm, and exposure of the sperm to reactive oxygen species is reduced. Compared with other methods, the procedure takes longer to perform and requires special media. Only a fraction of the motile sperm is recovered, thus this may not be a good preparation when sperm concentration or motility is reduced. Motile sperm concentration in the final sample often exceeds 90%. While the density-gradient and swim-up methods do a good job of providing a "clean" final sample, no preparation can guarantee a final sample completely free from infectious agents or cellular debris.

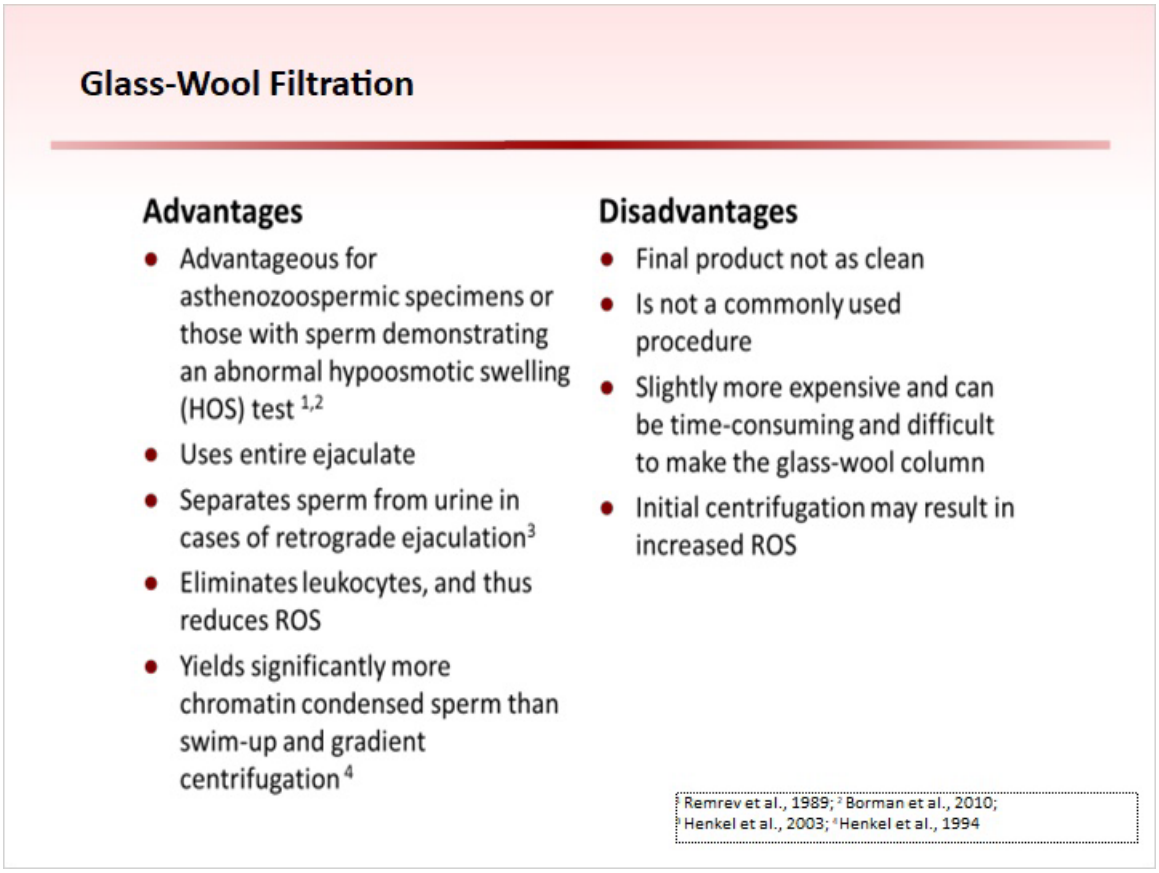
## *1.20 Column Filtration Method*




**Notes:**

Column filtration methods are based on the fact that dead or dying sperm are sticky and will attach to glass-wool or dextran gel beads even in the presence of high protein concentrations. The preparation of the column can be somewhat difficult, and with glass-wool columns, it is important to rinse the column thoroughly to remove loose glass-wool fibers. These methods also require an initial wash centrifugation of the semen, with layering of the washed and diluted pellet onto the column for a second centrifugation step.

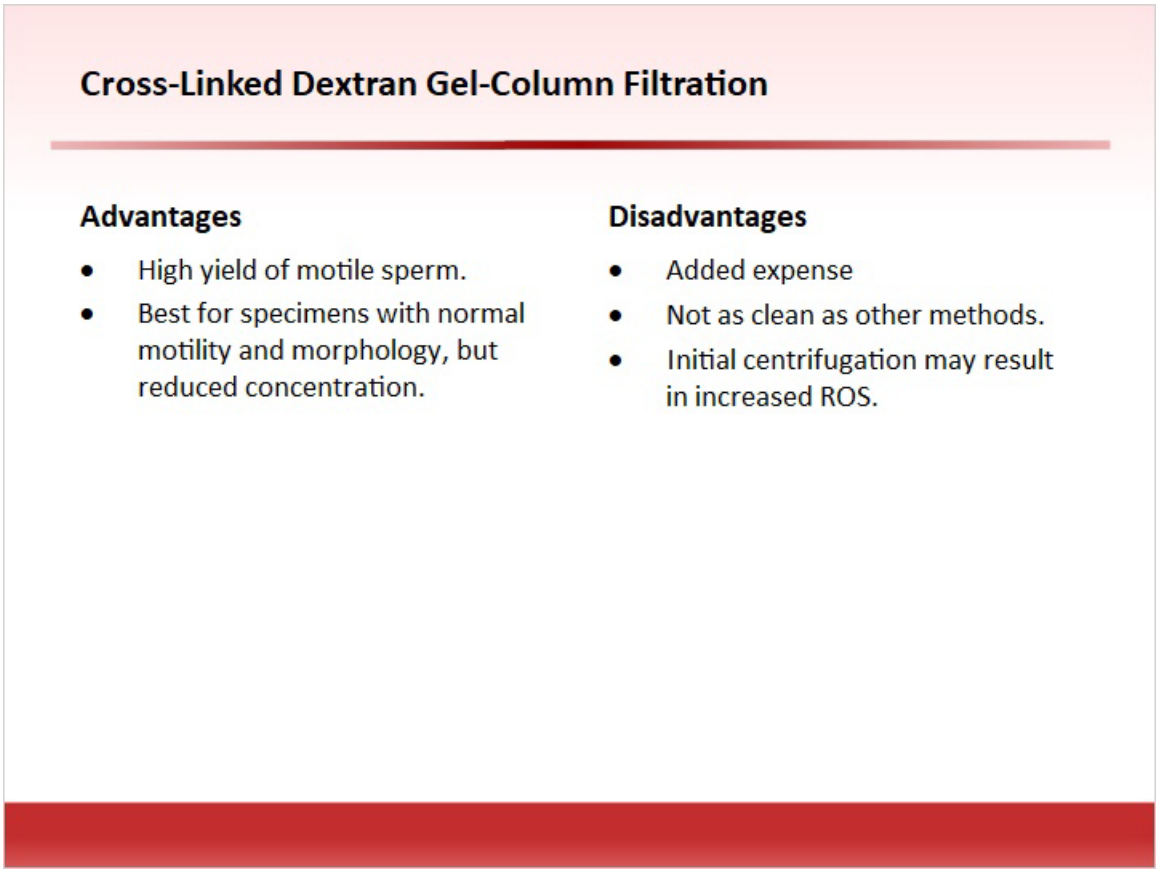
## *1.21 Glass-Wool Filtration*




**Notes:**

Glass wool filtration results in a similar recovery of motile sperm as in the density-gradient centrifugation method, yielding an average of 50%-70% progressively motile sperm. It is most successful for specimens with a high percentage of sluggishly motile sperm, or low total motility (asthenozoospermia), or where viability is low by the hypoosmotic swelling (HOS) test. Glass-wool filtration also uses the entire ejaculate, thus increasing the probability of recovering more sperm, removes leukocytes, and effectively separates sperm from urine following retrograde ejaculation. However, glass-wool filtration results in a sperm fraction that may not be as clean as other methods, particularly with its risk of contamination with glass-wool fibers. The set-up is more costly and time-consuming to prepare the columns. All in all, glass-wool filtration column filtration is not a commonly used procedure for sperm preparation for ART.

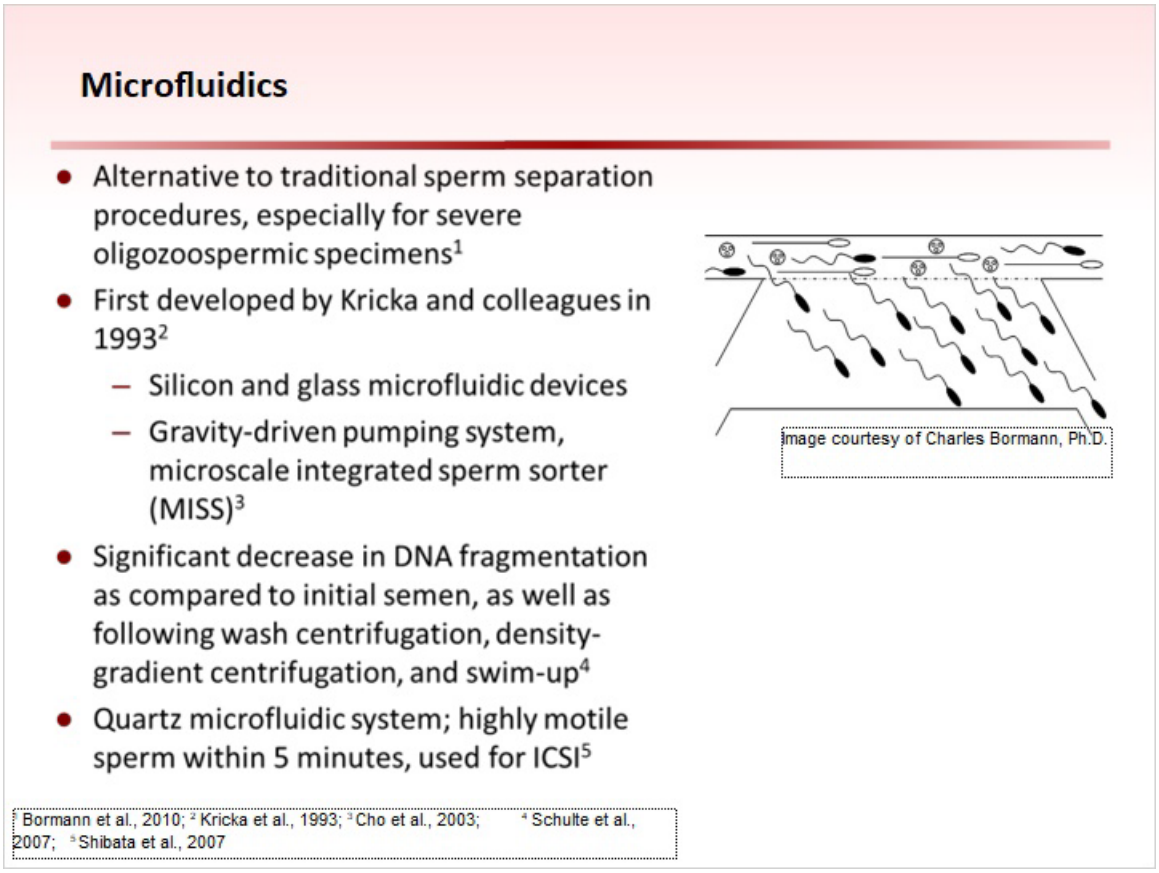
## *1.22 Cross-Linked Dextran Gel-Column Filtration*




**Notes:**

Cross-linked dextran gel-column filtration is based upon the same principles as the glass-wool filtration columns. This type of filtration results in a high yield of motile sperm, and is more advantageous for specimens with normal motility and morphology but reduced concentration. A disadvantage is the added expense, time, and technique for preparation of the columns, and the resulting fraction again may not be as clean as other methods, particularly the density-gradient centrifugation and swim-up methods.

## *1.23 Microfluidics*



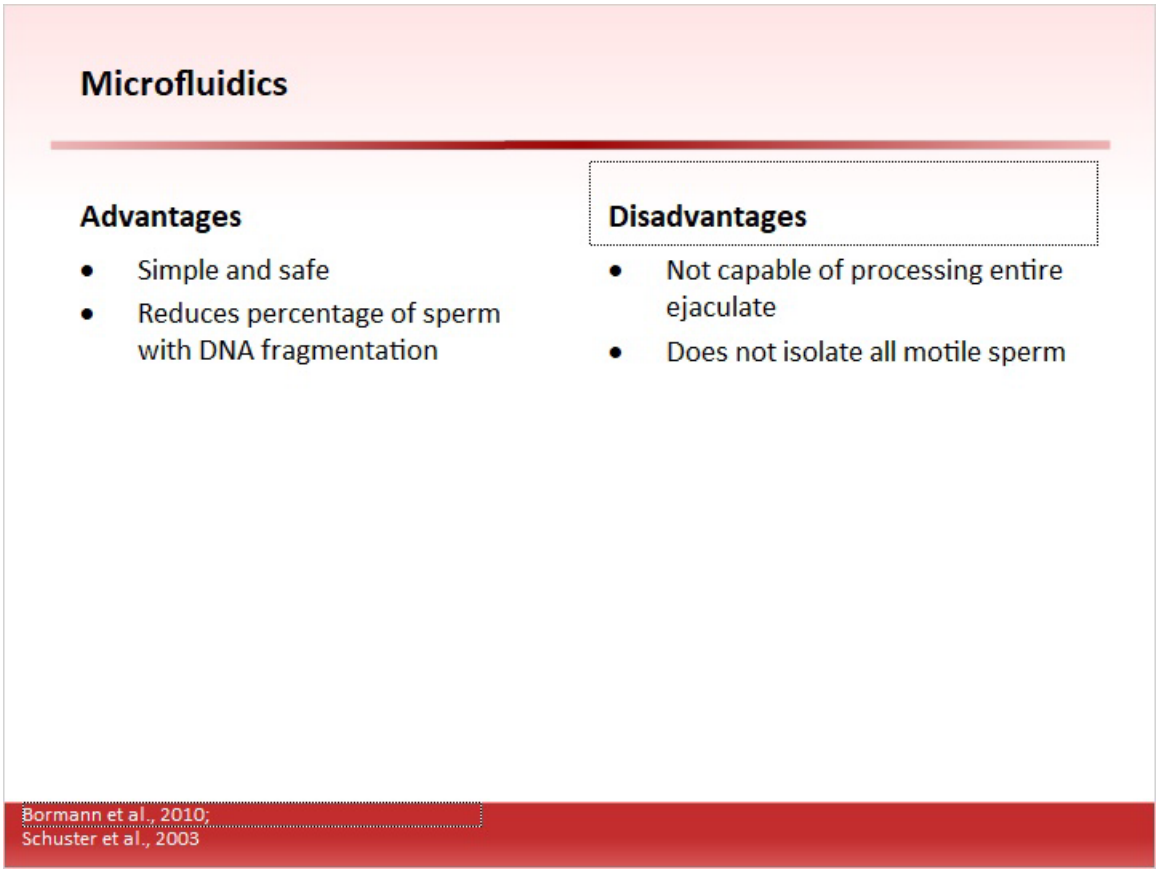

**Notes:**

The most common sperm preparation techniques are suboptimal for patients with severe oligozoospermia, since many of these specimens exhibit not only low sperm count but large amounts of cells and debris. Microfluidics has been proposed as an alternative to traditional sperm separation methods, especially in suboptimal, oligozoospermic semen specimens. Kricka and colleagues in 1993 first developed microfluidic devices using silicon and glass devices for sperm isolation. Such devices use a network of branching microchannels that separate or characterize sperm based upon motility and forward progression. More recently, Cho and colleagues developed a gravity-driven pump system, termed a microscale integrated sperm sorter, or MISS. These systems contain inlet/outlet ports, fluid reservoirs, gravity-driven power sources, and converging microchannels with laminar flow. All components then facilitate sperm sorting. Nonmotile sperm, cells, and debris are moved into the waste reservoir.

A more recent study compared microfluidic sperm sorting with wash centrifugation and density-gradient centrifugation. This group demonstrated a significant decrease in sperm with DNA fragmentation. In a

quartz microfluidic system, a high percentage of highly motile sperm were separated within 5 minutes and used for ICSI, and a 50% fertilization rate was achieved.

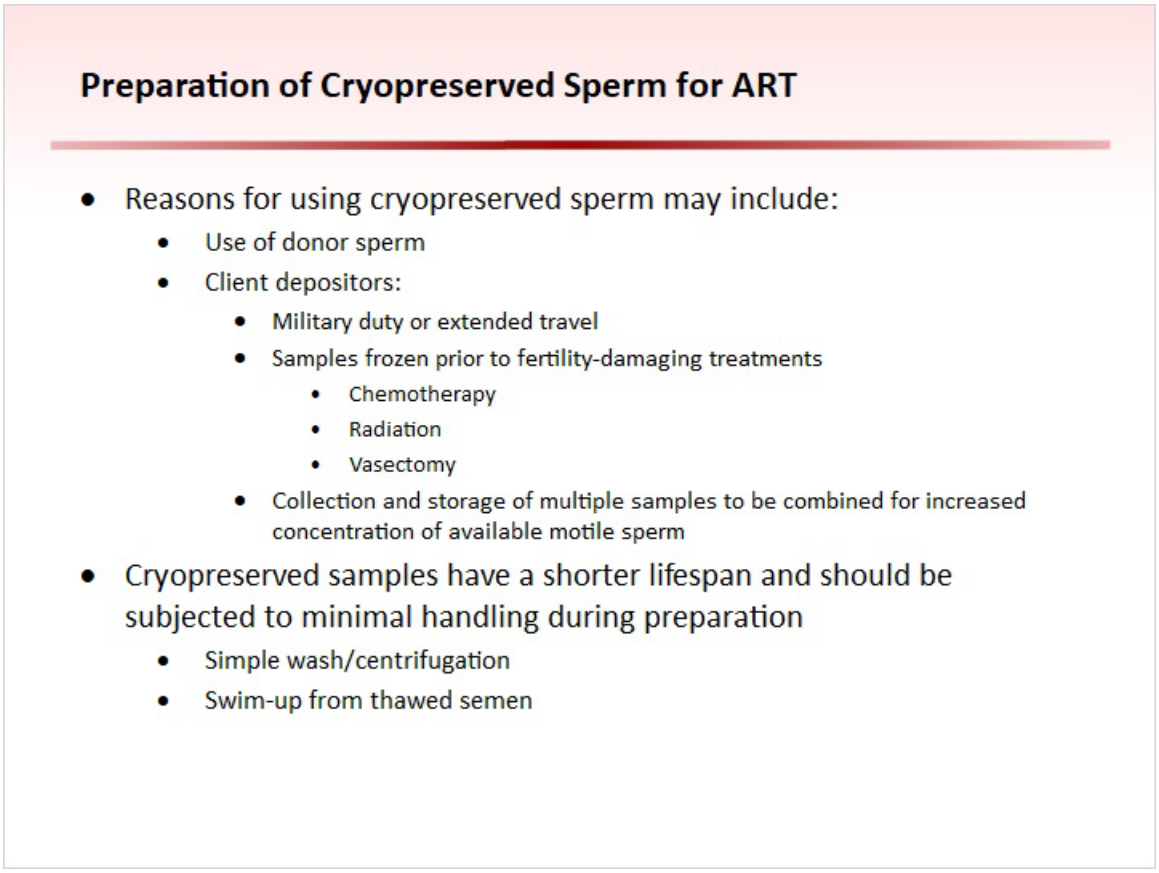
### *1.24 Microfluidics*




**Notes:**

Microfluidic separation systems are simple and safe, and result in motile sperm with normal morphology and reduced DNA fragmentation as compared with traditional methods. However, microfluidics devices are not capable of processing an entire ejaculate, and thus do not isolate all motile sperm. However, adequate numbers of sperm for use in ICSI can be easily obtained in a short period of time from severely oligozoospermic specimens. Microfluidic devices for sperm separation continue to be developed and tested, and are not yet in the mainstream of standard sperm separation procedures.

## *1.25 Preparation of Cryopreserved Sperm for ART*

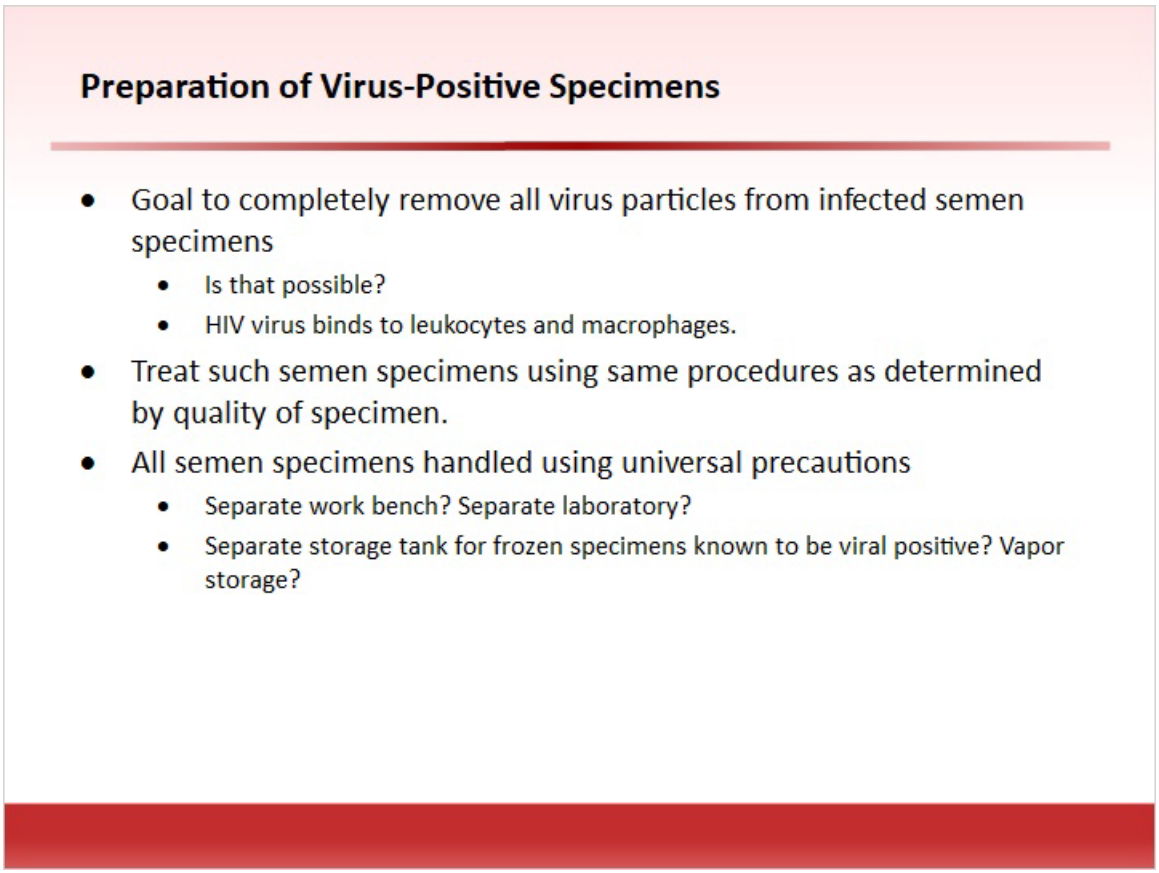



**Notes:**

Patients may choose to use cryopreserved semen samples for ART for many reasons, including use of donor sperm (which must be cryopreserved to comply with US Food and Drug Administration [FDA] regulations) or for those with military duty or extended travel. Some have samples frozen prior to fertility-damaging treatments such as chemotherapy, radiation and vasectomy. When working with cryopreserved samples, it is important to realize that samples will inevitably have a lower number of motile sperm than they do prior to freezing. As a result, patients should be counseled on how many vials or units of sperm to use based on post-thaw analysis. Most often, patients will purchase at least 2 vials of donor sperm, or request 2 vials of client depositor semen for an ART cycle so that adequate numbers of motile sperm can be recovered for insemination.

When preparing cryopreserved samples for ART, minimal handling is best, as cryopreserved samples are less robust than fresh samples. A single centrifugation basic wash is typically adequate to remove seminal fluids and cryoprotectants. It is also important to dilute the thawed semen cryopreservative slowly, so as to avoid osmotic shock to the spermatozoa.

## *1.26 Preparation of Virus-Positive Specimens*

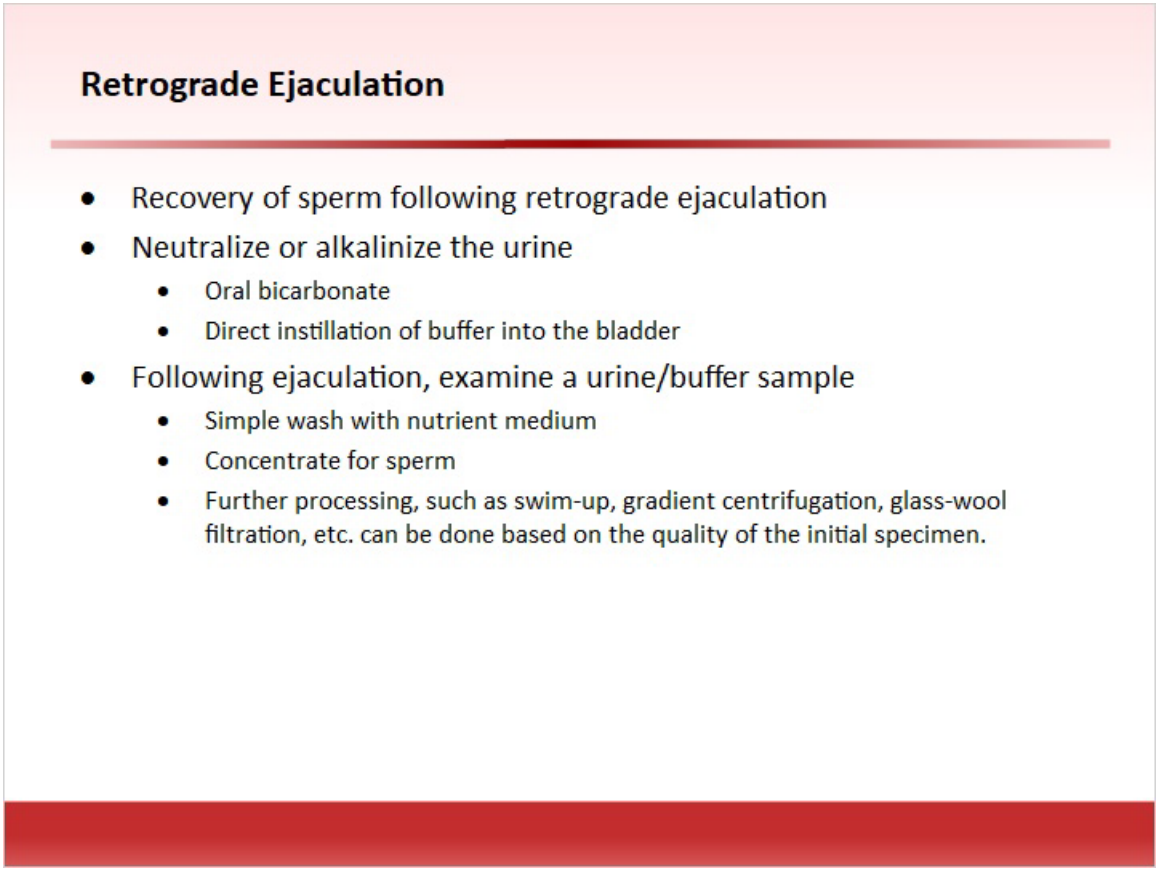



**Notes:**

With improvements in antiviral treatments and longer survival rates, there are more patients who are positive for HIV or hepatitis who are seeking assisted reproductive treatments. Andrology and embryology laboratories should be well prepared to process both sperm and oocytes from virus-positive patients. The methodology for sperm preparation for the virus-positive male would be determined based on the quality of the initial fresh or frozen semen specimen.
Ideally, the goal of sperm preparation for ART would be removal of all virus particles from infected semen specimens. However, this is not really possible with current methodology. Virus-positive specimens should be handled with universal precautions in the same manner that any biological specimen would be handled. Suggestions include using a separate work bench in the laboratory, separate incubator, and separate centrifuge, with decontamination after each use. At a minimum, known virus-positive semen specimens that are frozen and stored for future use must be housed in

impenetrable containers such as cryogenic storage straws and/or stored in the vapor phase of liquid nitrogen.

### *1.27 Retrograde Ejaculation*

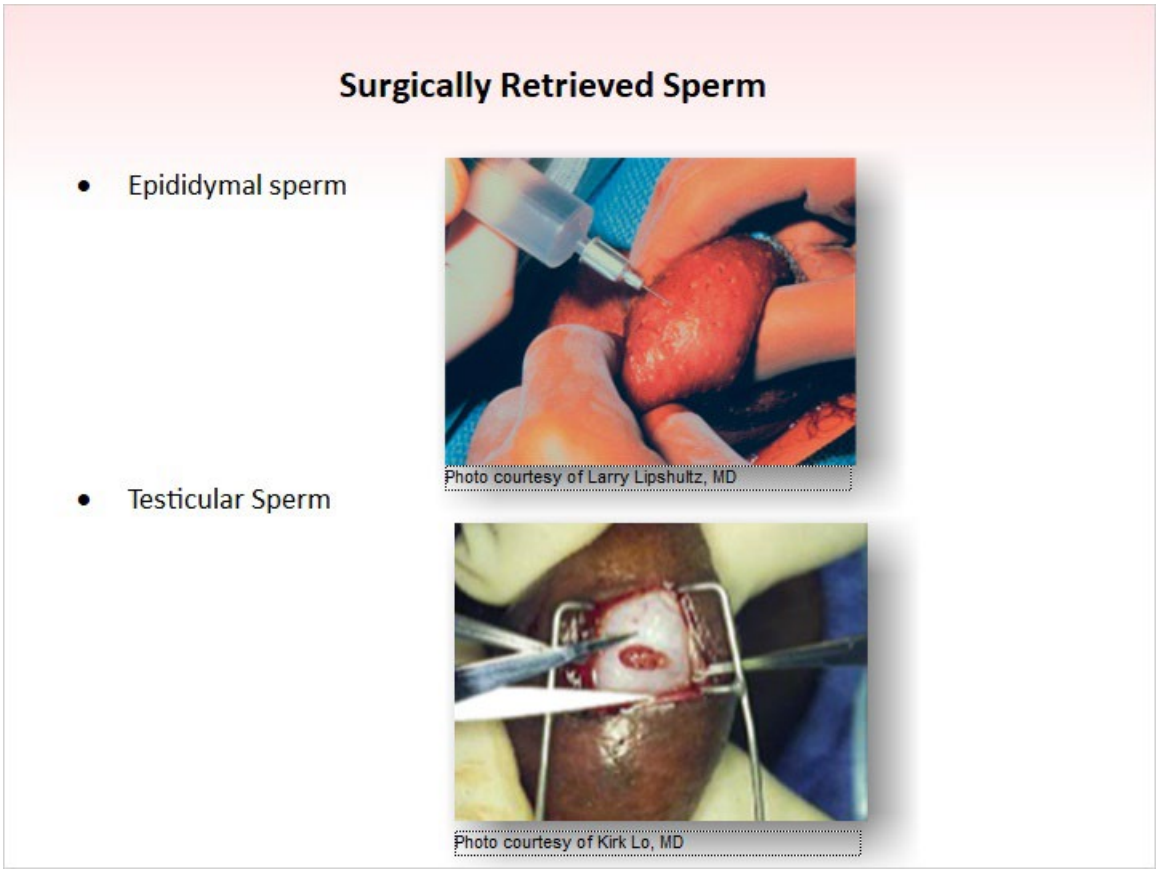



**Notes:**

With certain medical conditions there may be no antegrade ejaculation, with semen passing in a retrograde fashion into the bladder. The acidic and hypoosmotic nature of the urine is detrimental to sperm. Sperm can be recovered from the urine following retrograde ejaculation and used for assisted reproduction. In order to recover viable sperm, the urine must be neutralized by oral bicarbonate treatment or by instilling appropriate buffer into the bladder using a sterile catheter. Following masturbation, any antegrade ejaculate should be recovered and processed. A urine sample should be produced within 5 minutes of ejaculation or orgasm, and should be analyzed. Based on the quality of the retrograde specimen (sperm concentration and motility), the urine will be washed free from the sperm using a simple wash technique. Other processing techniques such as swim-up, density gradient centrifugation, or glass-wool filtration can be done based

on the quality of the specimen and the end use of the sperm in ART.

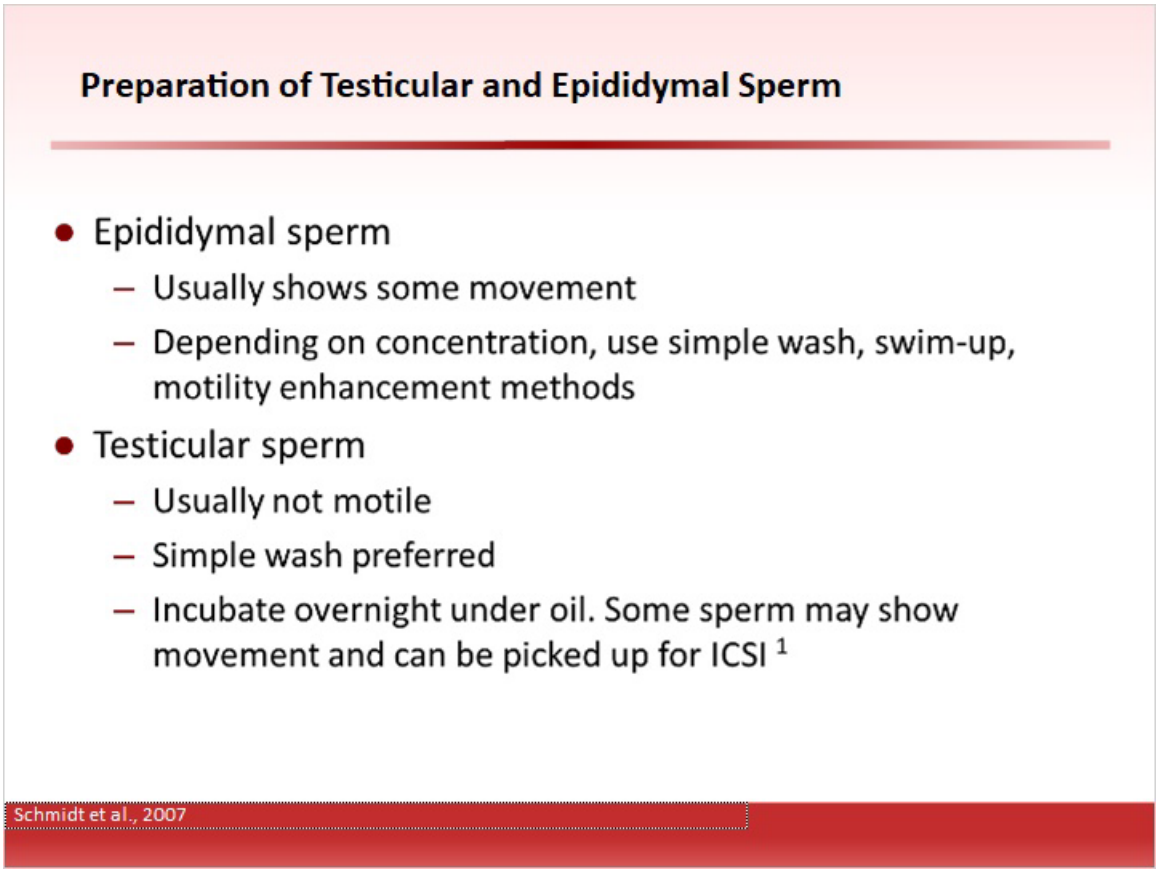
### *1.28 Surgically Retrieved Sperm*




**Notes:**

Sperm can be surgically retrieved from either the epididymis-termed microepididymal sperm aspiration (MESA)-or retrieved directly from the testicle, known as testicular sperm extraction (TESE). It has been more than 20 years since reports of the first ICSI procedure using sperm retrieved directly from the testicle. Not only has ICSI become a standard procedure, but TESE has been routinely used for males with obstructive and nonobstructive azoospermia.

## *1.29 Preparation of Testicular and Epididymal Sperm*




**Notes:**

Epididymal aspirates are usually very low in volume and must be carefully manipulated. Sperm from the epididymis may show twitching or slow movement. Depending on the initial analysis, epididymal sperm can be washed by simple centrifugation or even swim-up. Often the concentration is low, and thus these specimens would be used for IVF/ICSI rather than intrauterine insemination. Epididymal sperm may also be good candidates for motility enhancement and selection by hypoosmotic swelling.
Sperm retrieved from testicular tissue generally are not motile and are few in number. The sperm may be teased from the tubules and washed by simple centrifugation. If frozen-thawed, sperm can be found in the fluid, or teased from the tubules in the tissue. It has been shown to be advantageous to incubate testicular sperm overnight under oil, which can result in a twitching movement, thus facilitating pickup of "viable," normal sperm for ICSI.

## *1.30 Surgically Retrieved Sperm for ART*

### Surgically Retrieved Sperm for ART

- Examine for presence of sperm in the operating room
- Meticulous laboratory search of mechanically processed testicular tissue, possibly to include enzymatic treatment
  - Chance of finding sperm after chemical digestion when sperm were not initially found is 25%–30%[1]
  - Other studies report a success rate of 7% using mechanical preparation followed by multi-hour, many-technician search in the laboratory. [1]

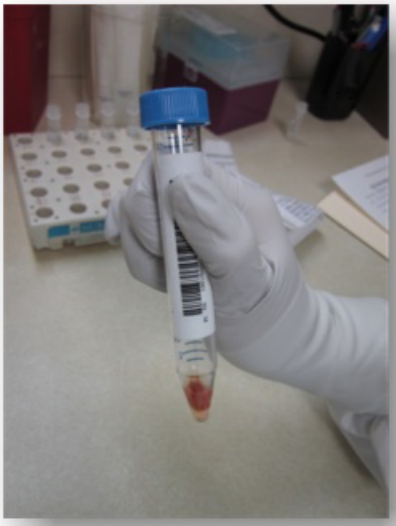


Photo courtesy of Grace Centola, PhD

Dabaja & Schlegel, 2013

**Notes:**

Often the biopsy tissue is examined in the operating room to determine the presence of sperm. The surgeon will continue to biopsy different areas of the testicle until sperm are found. However, sperm are not always identified in the operating room. A meticulous laboratory-based search of mechanically processed tissue, including enzymatic treatment, can be performed to increase the chances of finding sperm. Some studies have reported a 25%-30% chance of finding sperm following enzymatic digestion, when sperm were not initially found after mechanical manipulation. Additional studies have reported a success rate of 7% when mechanical preparation is followed by multi-hour, multi-technician search for sperm.

## *1.31 Cryopreservation of Surgically Retrieved Sperm*

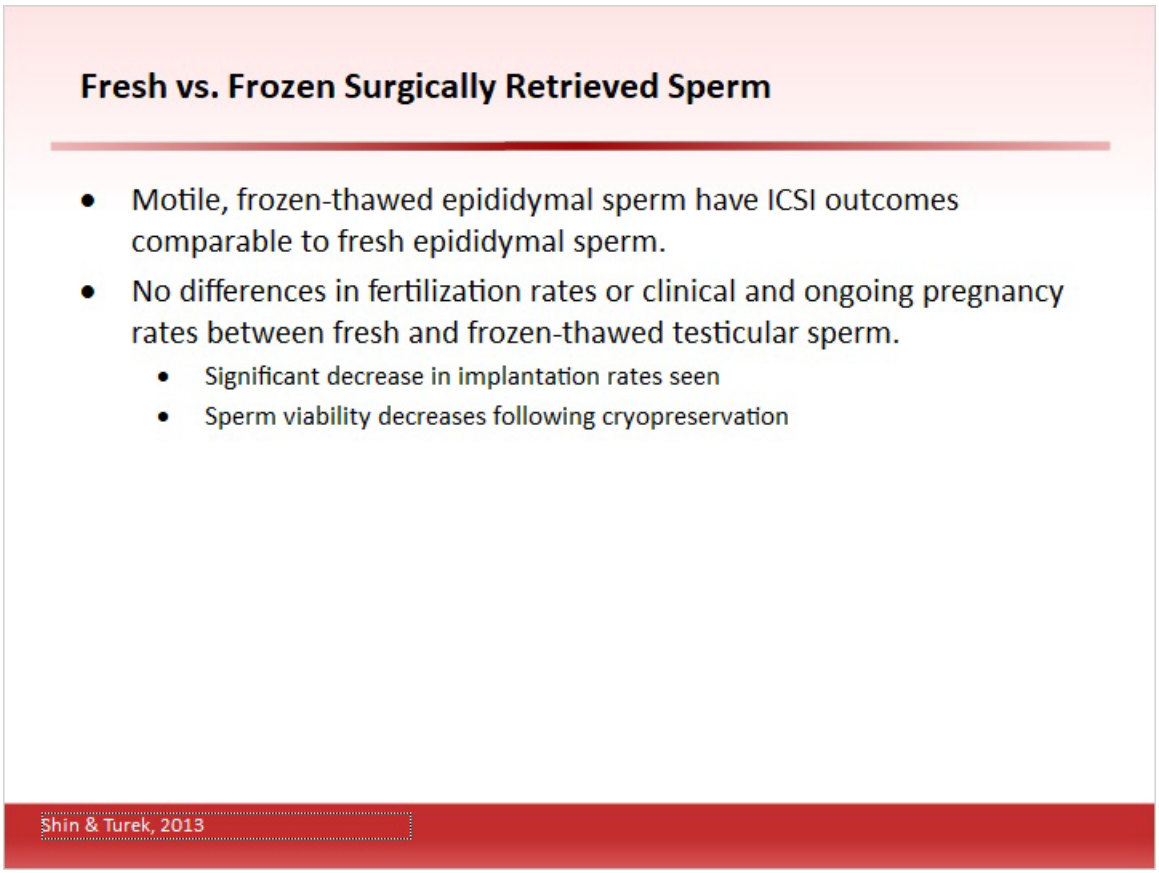
### Cryopreservation of Surgically Retrieved Sperm

- Epididymal aspiration: microsurgical (MESA), percutaneous (PESA)
  - Must be kept in isotonic media capable of supporting the sperm until processed
  - Synthetic HEPES-buffered human tubal fluid (HTF) supplemented with 0.5% human serum albumin (HSA) and antibiotic
  - HEPES-buffered Ham's F-10 with HSA
- Surgical retrieval: Testis biopsy (TESE), microdissection TESE, testis fine-needle aspiration (FNA)
  - Tissue is minced or seminiferous tubules teased apart using needles. Tubule contents squeezed into surrounding buffered medium such as HEPES-buffered HTF media
  - Sperm removed, used immediately or stored for future use
  - Sperm will not be progressively motile, but may be twitching
  - Freeze tissue pieces separately from cell suspension
  - Standard TEST-yolk-glycerol cryopreservative

**Notes:**

Both epididymal aspirates and testicular tissue can be cryopreserved so that sperm might be available at a later date and for multiple IVF cycles. Surgical retrieval with cryopreservation eliminates the need for surgery on the day of oocyte retrieval. Epididymal aspirates and testis tissue cryopreservation pose unique requirements for cryopreservation. Aspirates must be kept in isotonic medium until the specimen can be transported from the surgical suite to the laboratory for processing. Such media include HEPES-buffered synthetic human tubal fluid supplemented with human serum albumin as a protein source, or other media such as Ham's F-10 medium. Likewise, testis tissue is placed into buffer at the time of retrieval for transport to the laboratory. The tissue is then minced, and seminiferous tubules can be teased apart from the larger tissue pieces. The tubules can then be compressed and squeezed to release sperm into the surrounding medium. Testicular sperm are generally not motile per se. A few twitching sperm may be found, but this is variable. Both the spermatozoa and testis tissue pieces are frozen using standard TEST-yolk-glycerol cryopreservative in separate containers.

## *1.32 Fresh vs. Frozen Surgically Retrieved Sperm*

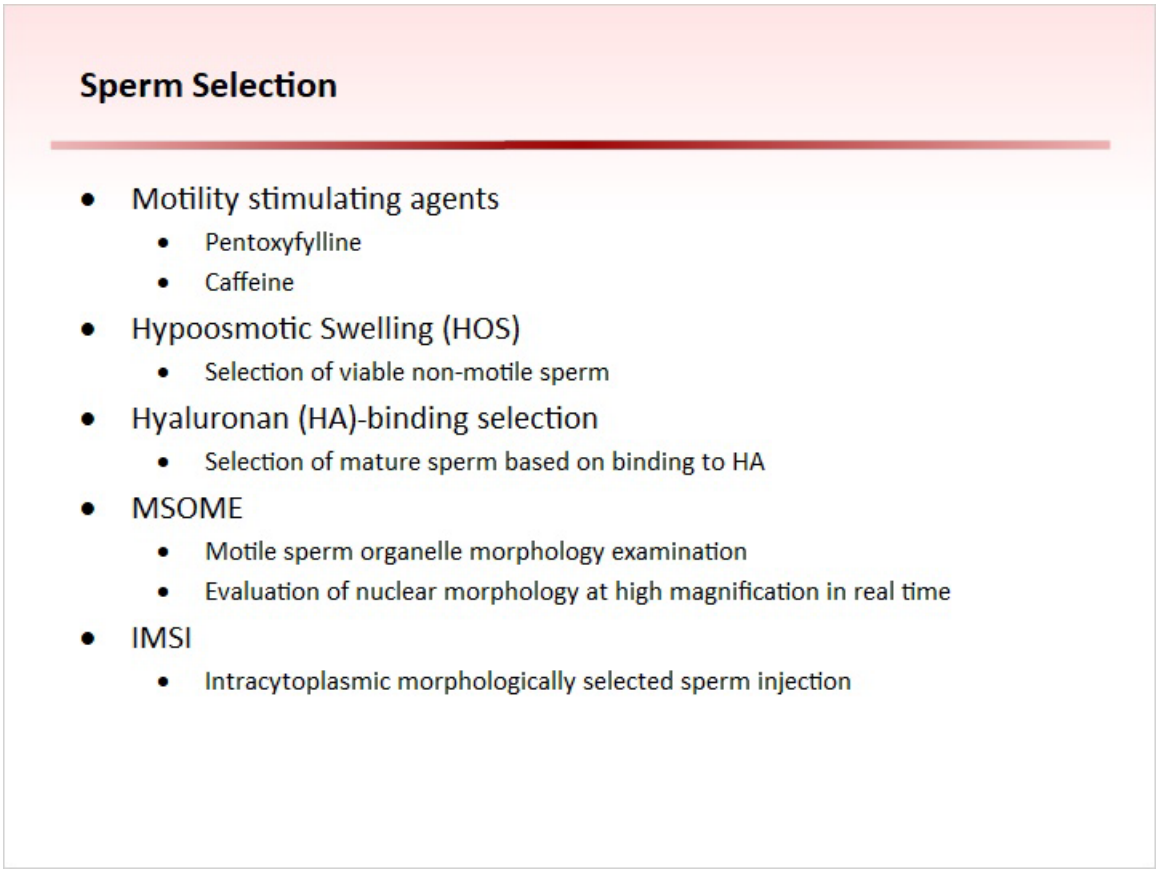



**Notes:**

Is fresh or frozen epididymal or testicular sperm better for ICSI?

Reports in the literature have generally concluded that there is no difference between fresh and frozen-thawed sperm. However, with cryopreservation and thawing, any motility is lost, and most centers prefer to work with motile sperm, since motility points to viability and some sense of normalcy. Nonmotile fresh testis sperm appear preferable for ICSI, since viability rates approach 90% in fresh testis sperm. A meta-analysis showed that fertilization rates, clinical pregnancy rates, and ongoing clinical pregnancy rates do not differ between the fresh and frozen groups. However, a significant decrease in implantation rates was observed with frozen-thawed testis sperm, presumably due to the decreased viability following thaw.

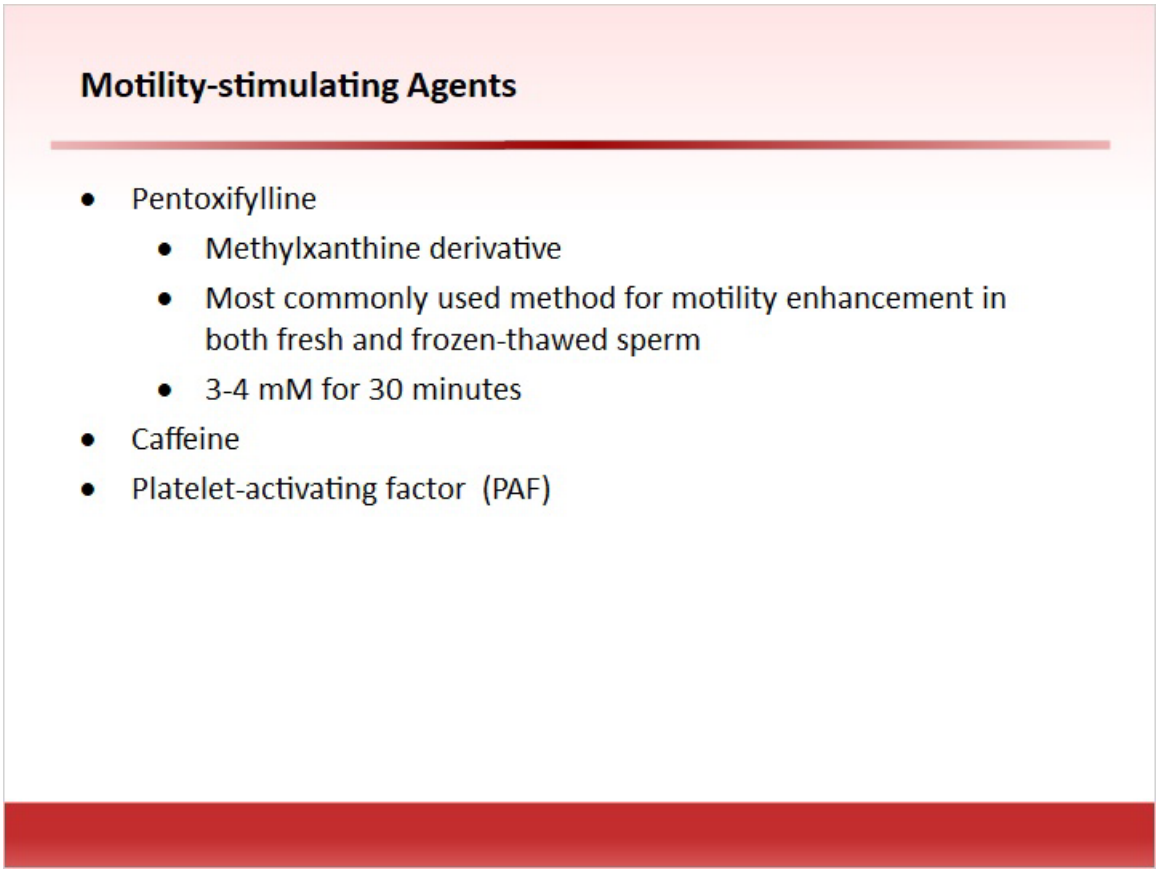
## *1.33 Sperm Selection*




**Notes:**

Once sperm are made available for IVF/ICSI, the choice of next steps are dependent on the quality of the sperm fraction. Certainly in cases where a good sperm concentration and motility are apparent, conventional techniques for oocyte insemination are chosen. Even if the concentration is low, but the morphology and motility are adequate, it is relatively easy to choose sperm for oocyte injection.
However, in the severely suboptimal specimen, such as severely oligozoospermic, asthenozoospermic, immotile, and teratozoospermic specimens, advanced sperm selection techniques may be warranted to assist in selection of an optimal sperm for ICSI. Advanced strategies include motility stimulation procedures and selection of sperm based on sperm maturity and ultramorphology as listed here.

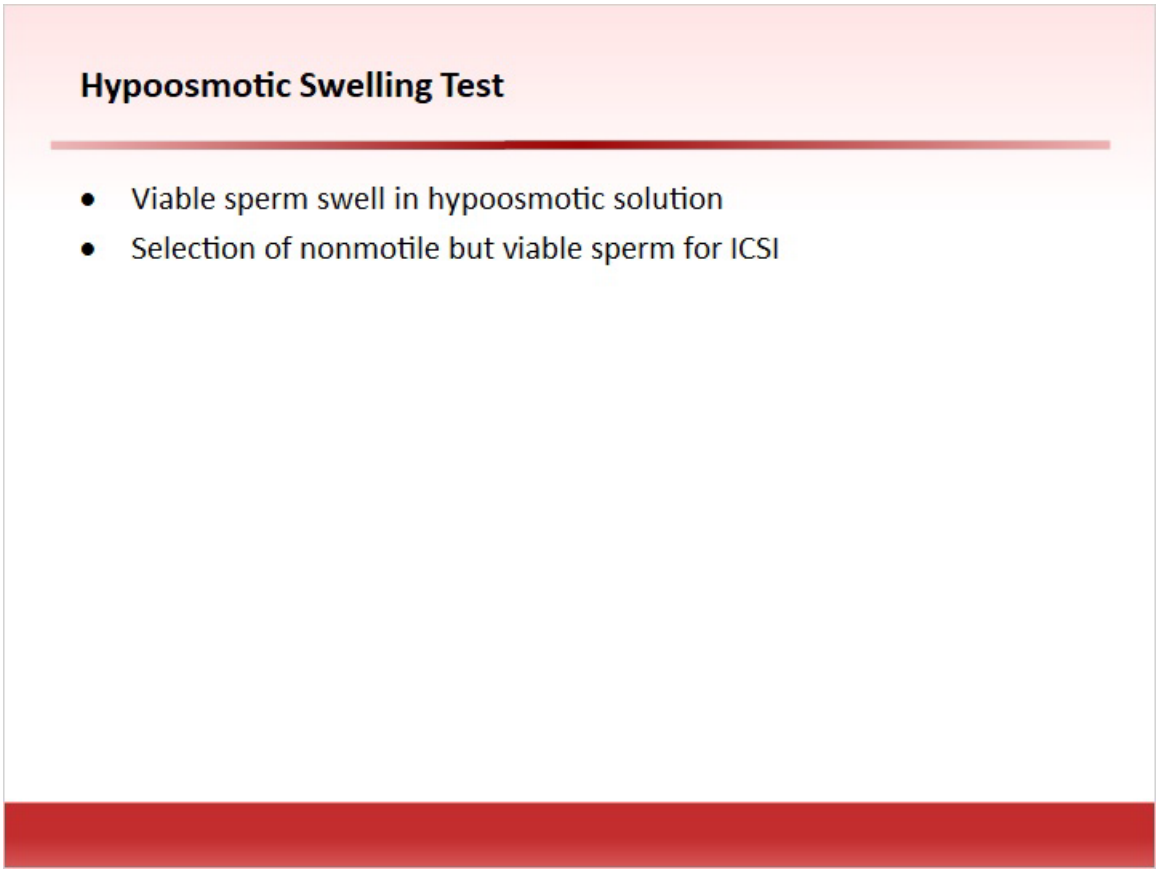
## *1.34 Motility-stimulating Agents*




**Notes:**

The standard method for determining sperm viability has always been sperm motility. However, in cases where sperm are retrieved from the testis, sperm are most often immotile. Frequently, cryopreserved sperm show decreased or no motility. Several chemicals are known to enhance sperm motility, most notably, pentoxyfylline and caffeine. Pentoxyfylline is a methylxanthine derivative that is an inhibitor of phosphodiesterase, which then increases intracellular levels of cyclic AMP (cAMP). cAMP stimulates motility, velocity, and hyperactivation of sperm, and has been shown to enhance the acrosome reaction. Interestingly, pentoxyfylline is approved by the FDA for treatment of vascular disease. The stimulant effects of caffeine can be used to improve or stimulate sperm motility. Platelet-activating factor also improves motility, and enhances sperm capacitation, the acrosome reaction and oocyte penetration. The mechanism of action of PAF is not completely understood and its use requires further study.

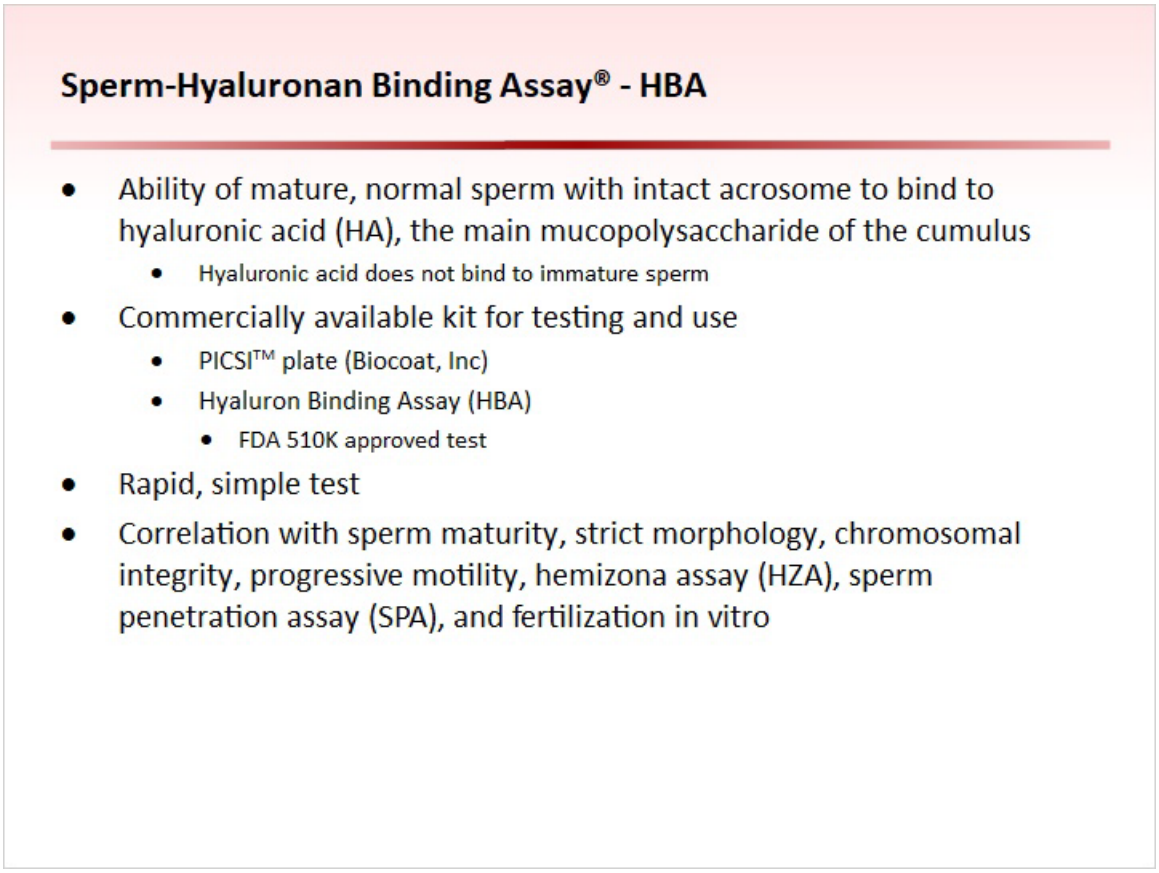
## *1.35 Hypoosmotic Swelling Test*




**Notes:**

Viable cells are able to maintain an osmotic gradient across the cell membrane. When sperm are placed into a hypoosmotic buffer solution, water will flow into the cell to maintain the intra- and extracellular osmotic balance. When water flows into the cell, it will expand in volume. Nonviable sperm will not exhibit swelling in a hypoosmotic buffer.
The sperm tail will swell or coil in such a buffer, and the viable sperm will thus be visible under phase contrast optics. The viable sperm can be picked up, washed from the hypoosmotic buffer, and used for ICSI.

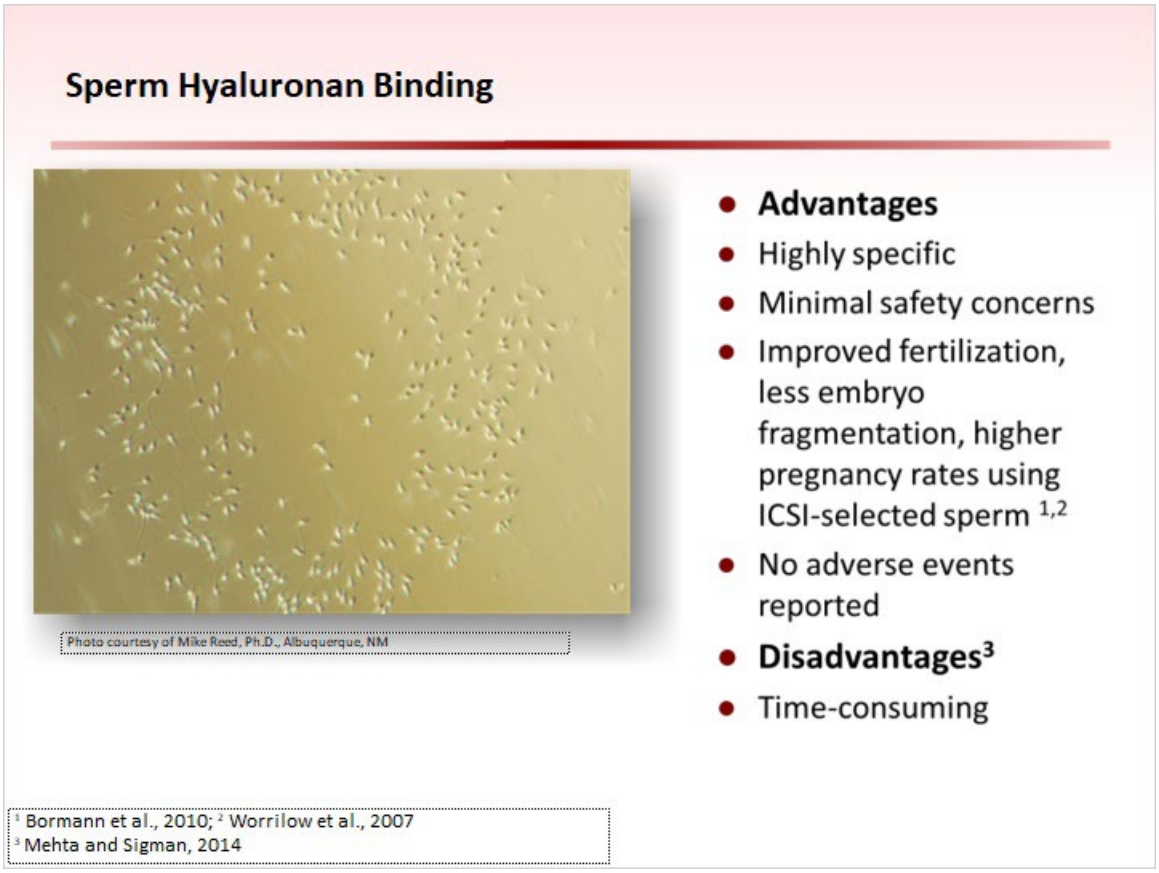
## *1.36 Sperm-Hyaluronan Binding Assay® - HBA*




**Notes:**

The Sperm-Hyaluronan Binding Assay® (HBA) is a commercially available sperm function test that determines the ability of the sperm to bind to a slide coated with hyaluronic acid. Live, mature sperm will bind to the hyaluronic acid coating the plate. These sperm also have been shown to be normal and with intact acrosome. Studies have shown that the HBA correlates with the level of sperm maturity, strict morphology, and chromatin integrity, as well as results of the HZA, SPA, and fertilization in vitro.

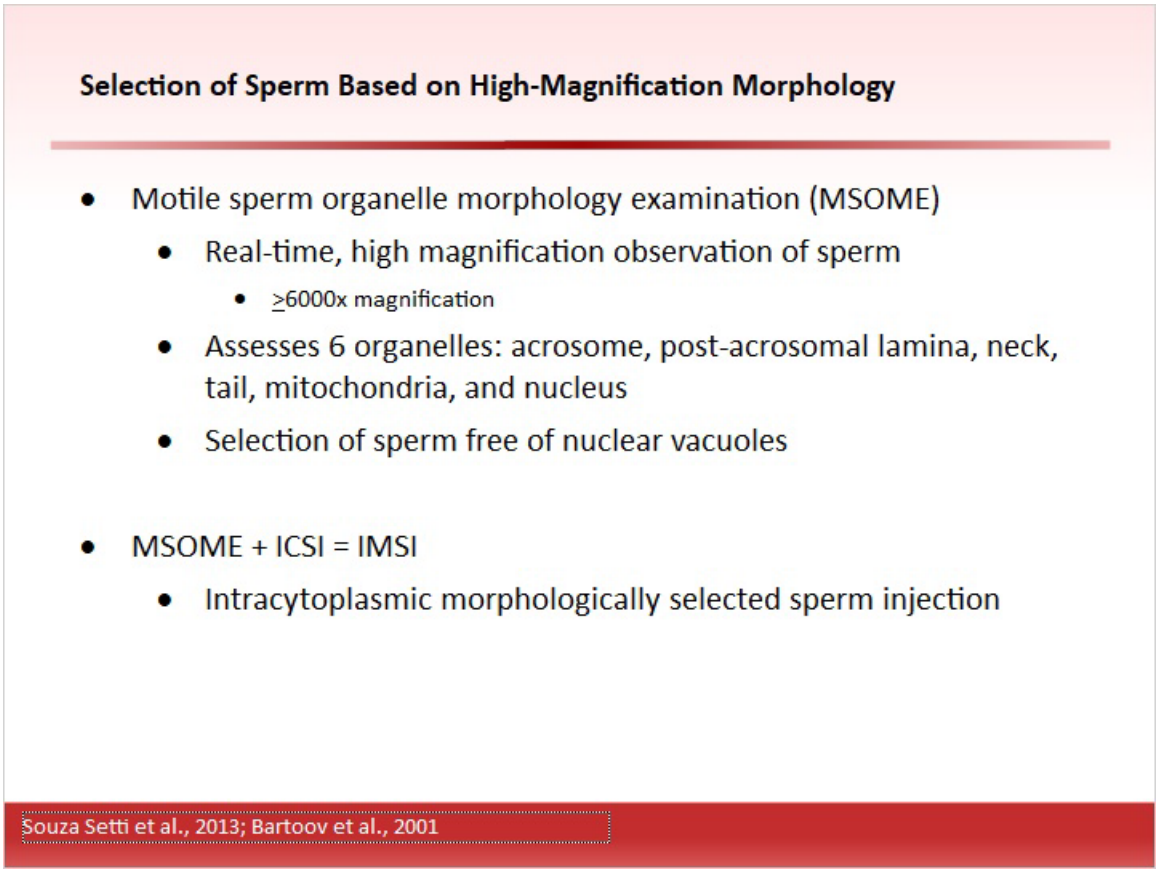
## *1.37 Sperm Hyaluronan Binding*




**Notes:**

This photomicrograph shows sperm binding to hyaluronic acid that coats a commercially available dish. Bound sperm can then be picked up by the embryologist and used for oocyte injection. One advantage of using this system for sperm selection is that the hyaluronan binding is highly specific, as only mature sperm bind. There are minimal safety concerns since hyaluronan is a naturally occurring component of cervical mucus, follicular fluid, and cumulus matrix. A study by Worrilow and colleagues reported a significant improvement in fertilization rates, less embryo fragmentation rates, and higher beta-hCG levels and pregnancy rates with hyaluronan-bound selected sperm. The miscarriage rate was also decreased when this system was used to select mature sperm for ICSI. A single disadvantage is the time commitment, particularly if multiple oocytes have been retrieved and need to be injected.

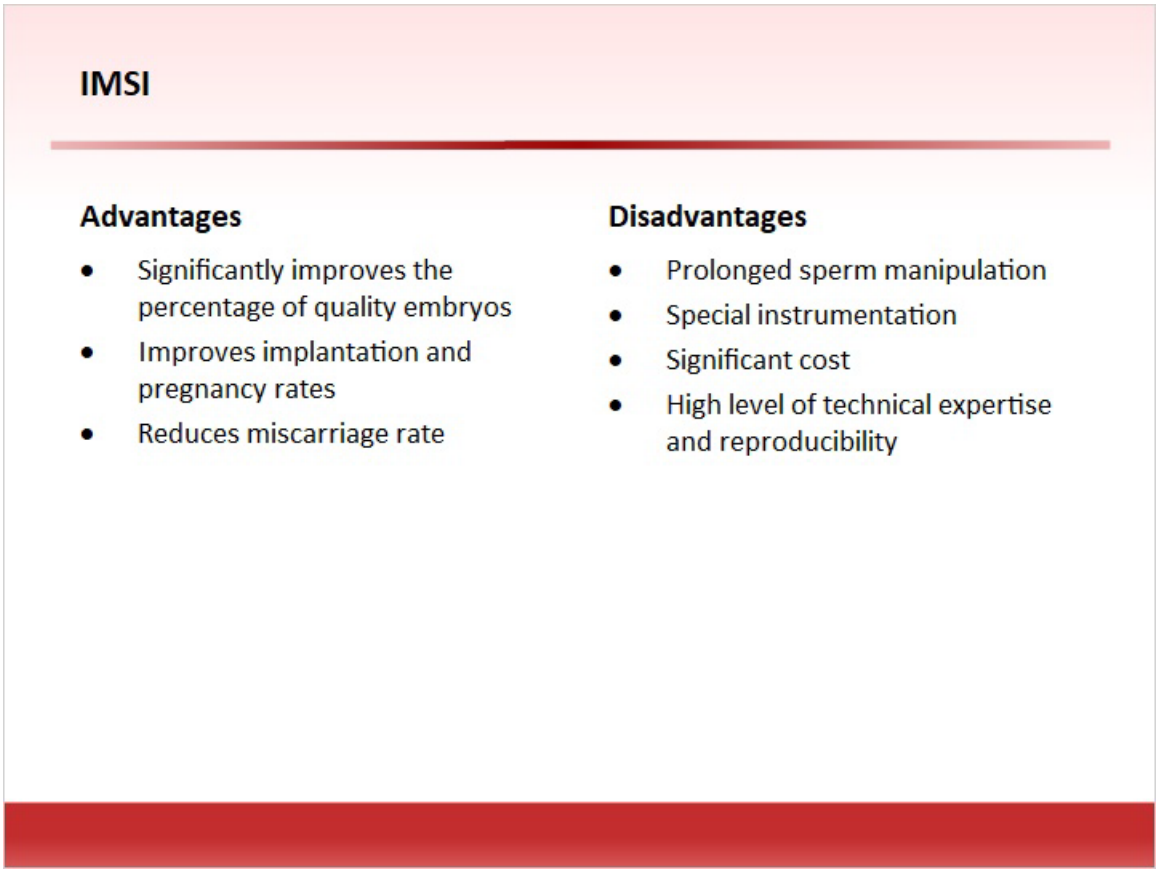
## *1.38 Selection of Sperm Based on High-Magnification Morphology*




**Notes:**

A new approach for selection of morphologically normal sperm using high magnification observation of unstained sperm was first reported in 2001 by Bartoov, termed motile sperm organelle morphology examination, or MSOME. Sperm can be visualized in real-time at magnifications of at least 6000x, which enables visualization of the sperm nucleus. MSOME allows selection of sperm free of nuclear vacuoles, which are related to impairment of embryo development. Together with micromanipulation systems, MSOME has allowed a modified ICSI procedure termed IMSI, or intracytoplasmic morphologically selected sperm injection.

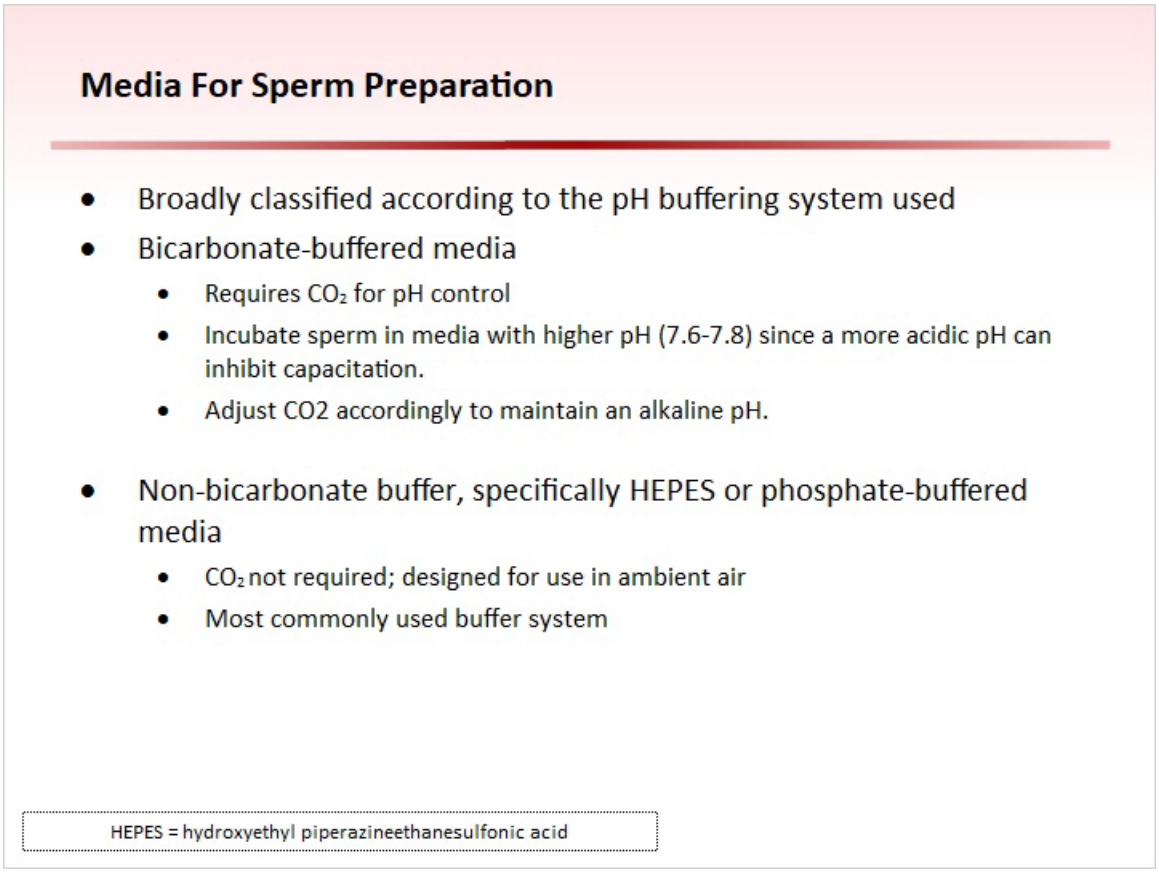
## *1.39 IMSI*




**Notes:**

There are advantages and disadvantages of IMSI. It has been proposed as an alternative to standard ICSI, especially in couples with repeated ICSI failures as well as those with increased DNA fragmentation. Some reports have shown improved implantation and pregnancy rates and decreased miscarriages rates after IMSI. Disadvantages include the need for prolonged sperm manipulation, special high-cost instrumentation, and the need for a high level of technical expertise. However, reports have been confusing and conflicting. Some studies have not shown significant differences between ICSI and IMSI. Clearly, more studies are needed to determine the utility of MSOME and IMSI in assisted reproduction.

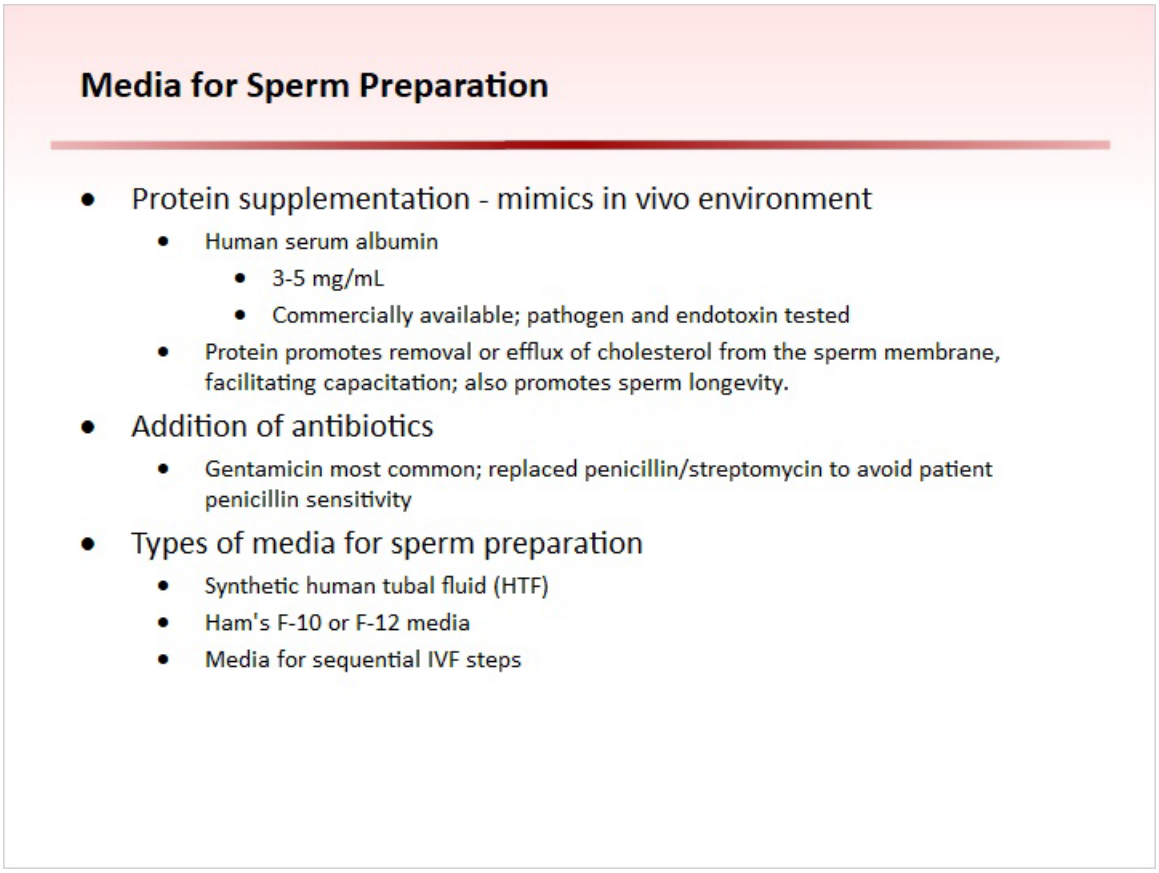
## *1.40 Media For Sperm Preparation*




**Notes:**

Media used for ART are usually classified according to the buffer system used to maintain the pH of the medium. Two buffering systems are commonly used with human gamete preparation. Bicarbonate-buffered medium relies on carbonic acid/bicarbonate equilibrium for maintaining pH. Bicarbonate-buffered medium requires carbon dioxide to maintain the pH with the weak acid, dissolved carbon dioxide. This medium is designed to maintain close to, or slightly below, neutral pH of 7.0 using the carbon dioxide environment. Non-bicarbonate media, specifically hydroxyethyl piperazineethanesulfonic acid (HEPES), are the most commonly used media for sperm preparation. Carbon dioxide is not required with this type of buffer to maintain the media pH. HEPES buffer has a working pH range of 6.8-8.2. The second most common buffer is phosphate, such as that used in phosphate-buffered saline where the pH is approximately 7.2-7.4. The greatest advantage to these buffering systems is that a carbon dioxide environment is not needed. The processing and incubation of washed sperm can be done in tightly closed tubes in ambient air.

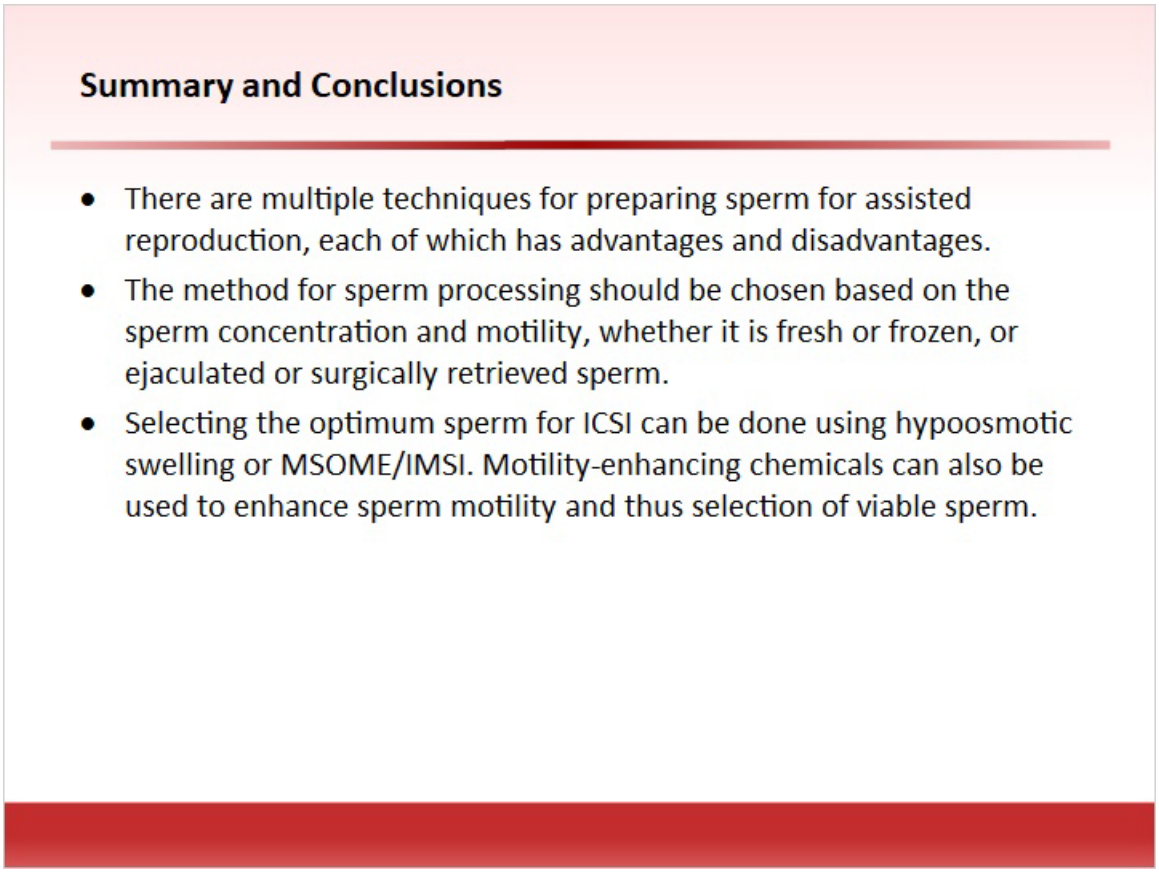
## *1.41 Media for Sperm Preparation*




**Notes:**

Protein supplementation of culture media for sperm preparation is essential for sperm preparation. Human serum albumin, purchased commercially, is the most common protein supplementation. Synthetic serum supplementation has been popular as well, especially for oocyte and embryo culture. Addition of protein helps to mimic the in vivo environment, and promote longevity of the spermatozoa. The protein is also known to promote efflux of cholesterol from the sperm membrane, thus facilitating sperm capacitation and ultimately the acrosome reaction.
Antibiotics are also commonly added to culture media for sperm preparation. The ejaculate commonly contains bacteria that are usually sensitive to antibiotics. The process of washing sperm in media with antibiotics should take care of any bacteria present in the semen or bound to sperm, prevent effects of bacteria on the sperm and oocytes, and prevent intrauterine infection after insemination or transfer.

## *1.42 Summary and Conclusions*




**Notes:**

There are several methods of choice for sperm preparation for assisted reproduction. These include simple washing, density-gradient centrifugation, filtration, and swim-up. The method chosen should be based on the quality of the sperm, specifically the sperm concentration and motility, whether the sperm are fresh or frozen, or whether ejaculated or surgically retrieved. Additionally, methods for sperm selection include use of hypoosmotic swelling buffer to choose viable sperm and high magnification morphological assessment termed MSOME/IMSI. Furthermore, motility-enhancing chemicals such as pentoxyfylline, platelet-activating factor, or caffeine can be used to improve or stimulate sperm motility and allow for selection of motile and thus viable sperm for assisted reproduction.

***1.43 Thank you!***


**Notes:**

Thank you for participating in this educational activity.