Sperm Selection

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Introduction

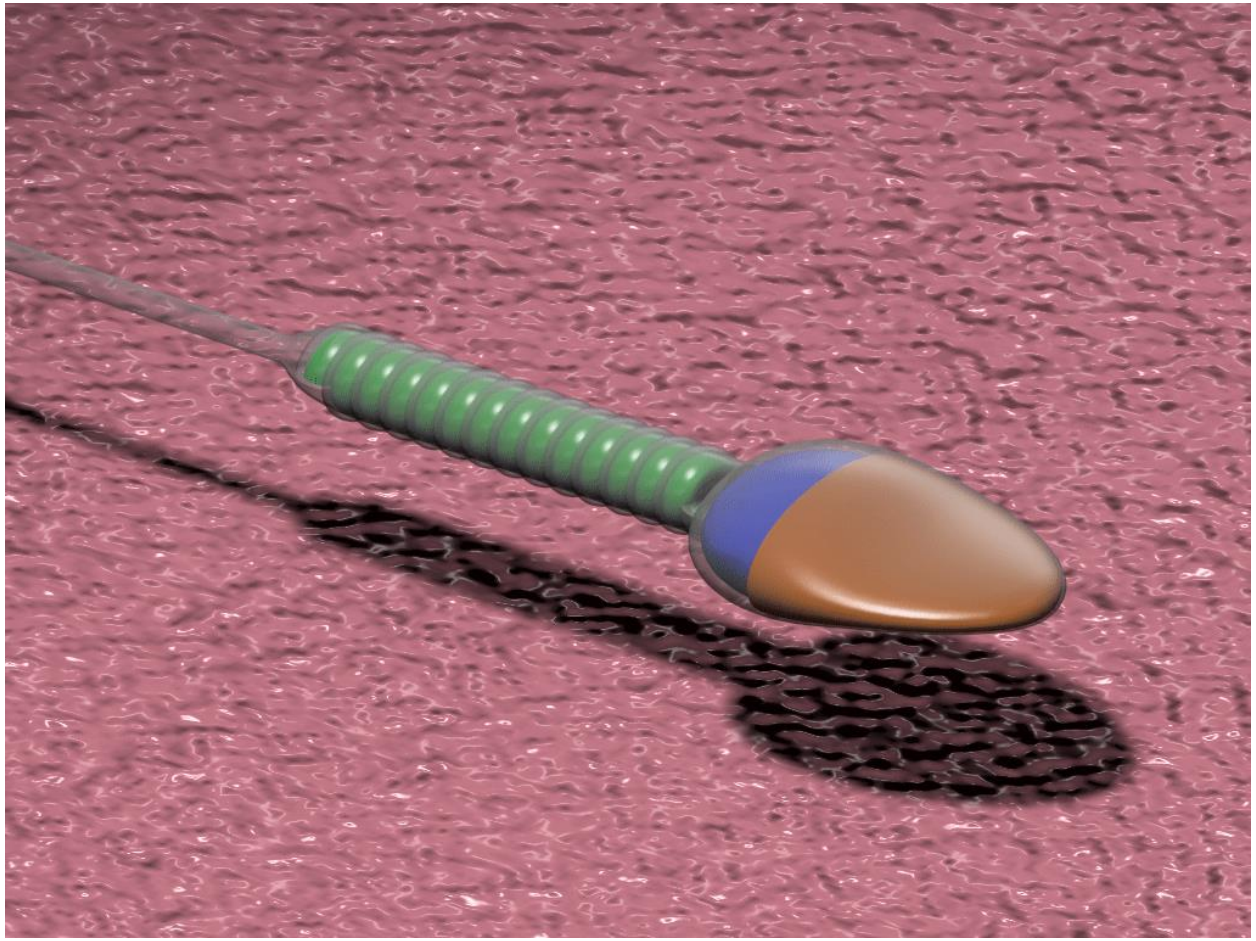
Male infertility is a factor in 50-60% of infertility cases.

Infertile men tend to have abnormal sperm parameters, such as;

- Low sperm concentration
- Poor motility
- Abnormal morphology
- Elevated levels of sperm DNA damage.
- Additionally, about 40-88% of sperm samples from infertile men have high levels of reactive oxygen species (ROS).

Agarwal A. et al, Reprod Biol Endocrinol. 2015

Pasqualotto FF, et al. Fertil Steril. 2000



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A high level of ROS and decreased levels of antioxidants can cause oxidative stress, which;

- Decreases sperm motility
- Decreases DNA integrity
- Decreases viability
- Increases mid-piece defects

Wright C,et al. Reprod Biomed Online. 2014

Poor DNA integrity is correlated with;

- Lower in vitro fertilization rate
- Lower pregnancy rate
- Irregular pre-implantation development
- Early loss of pregnancy
- Increased disease rates

Aitken RJ, et al. Reprod Biomed Online. 2007

- **The current sperm processing techniques suffer from a number of limitations:**

- **Not resemble** the natural process in vivo.
- **Contamination** with poor motile cells.
- Presence of **leukocyte** contamination.
- **Iatrogenic injury** due to prolonged exposure and oxidative stress due to centrifugation.
- Further visual inspection by the **embryologist**.

Sakkas D. Fertil Steril. 2013

Conventional Sperm Selection Methods:

- Simple Sperm Wash
- Swim-Up
- Density Gradient Centrifugation

Simple Sperm Wash

- The one-step wash technique does remove or reduce any cellular component such as the number of leukocytes, immature spermatozoa, or other cellular debris.

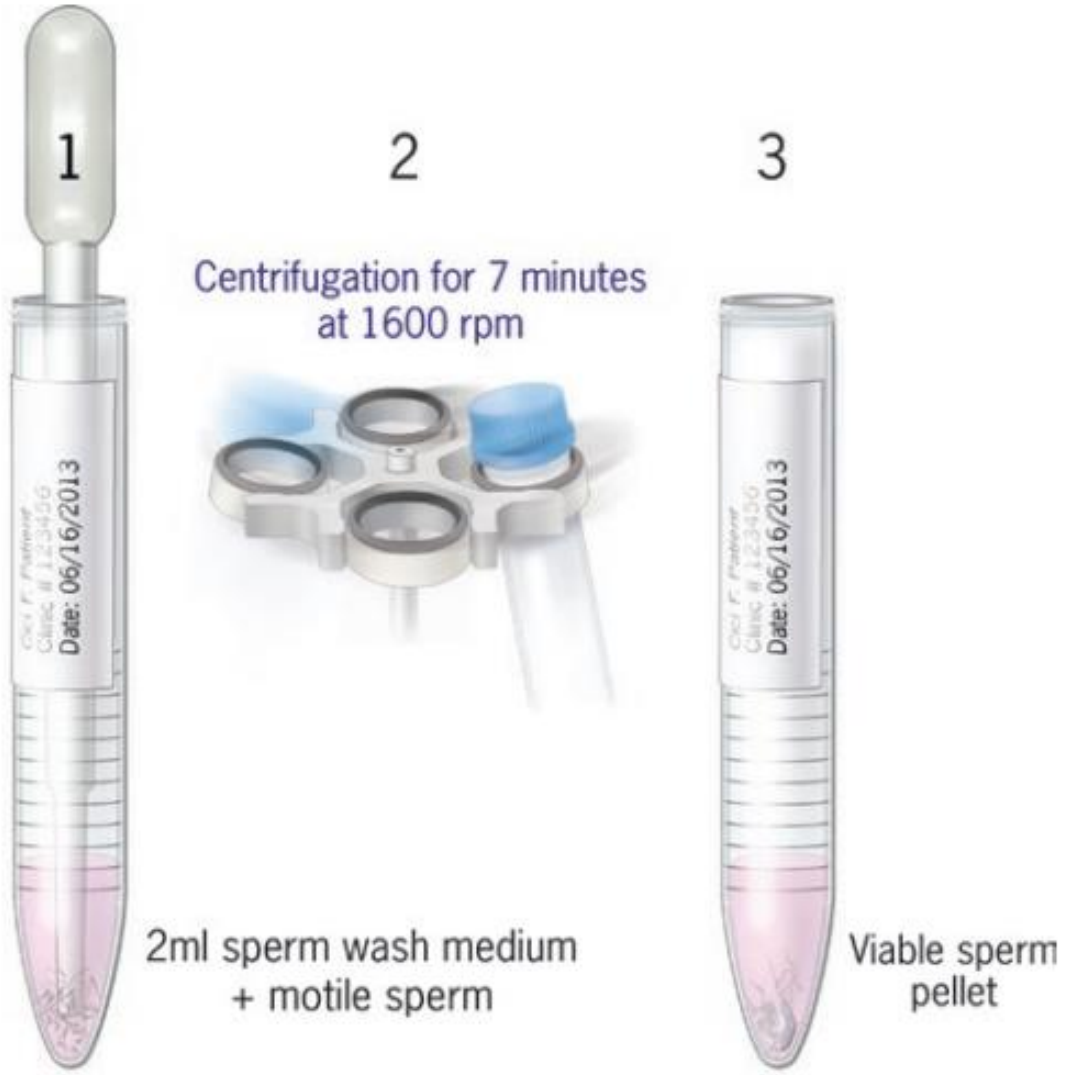
- It only removes the seminal plasma.
- Centrifugation causes additional harm by formation of reactive oxygen species (ROS) by abnormal spermatozoa and leukocytes.

Björndahl L, et al. Cambridge University Press; 2010.

Increased levels of ROS result in:

- DNA damage in spermatozoa
- Decreased sperm motility
- Increased numbers of apoptotic spermatozoa
- Decreased sperm plasma membrane integrity

Agarwal A, et al. Am J Reprod Immunol. 2008



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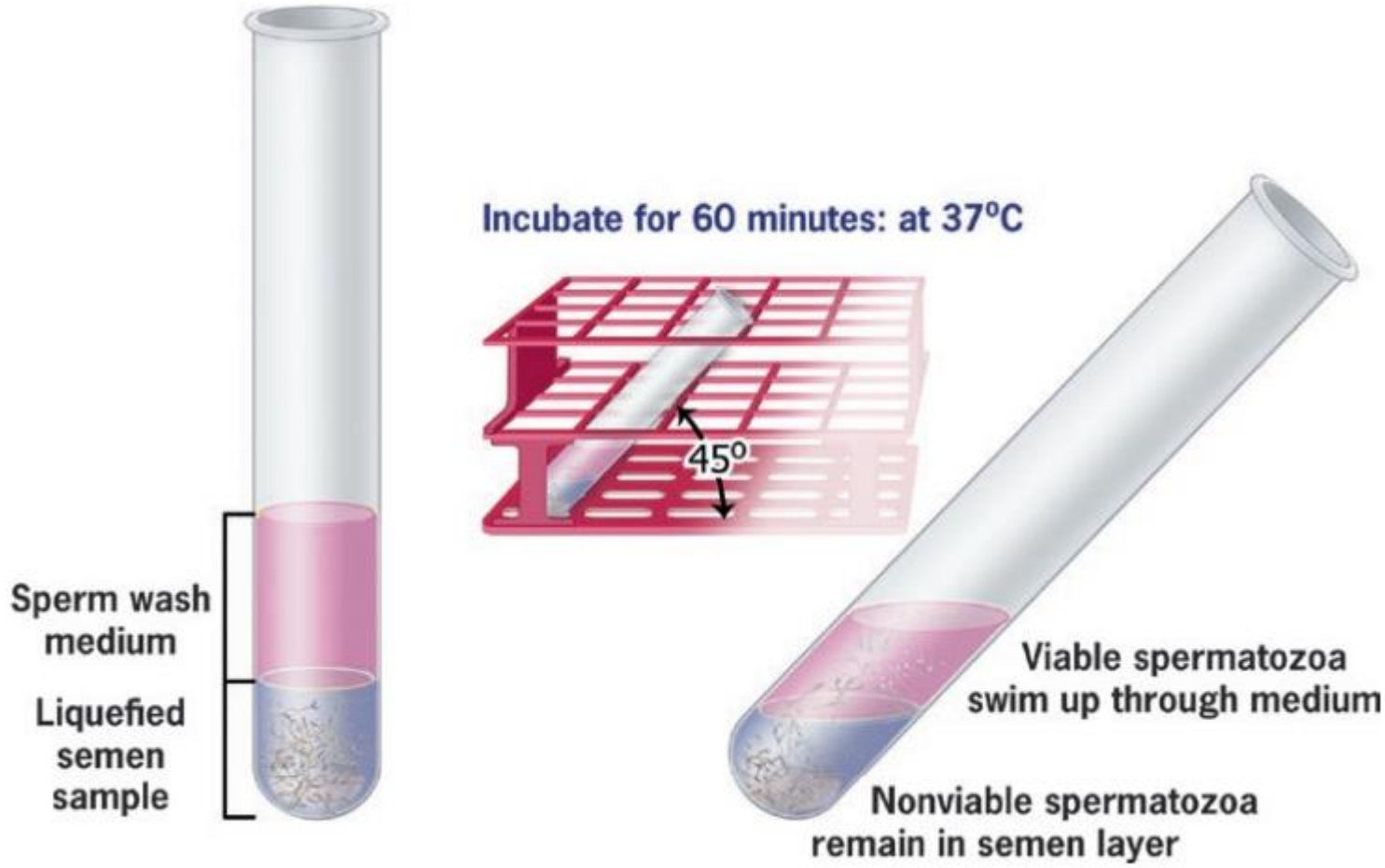
Swim-Up

- Swim-up method is inexpensive, and highly motile sperm can be obtained.
- The disadvantages are that the sperm recovery is relatively low. Only 5 to 10% of sperm cells are retrieved. When a pellet is used, sperm are trapped in the pellet and may not move into the clear medium.
- In addition, centrifugation results in the generation of ROS. Furthermore if the sample is contaminated with leukocytes, the close cell-to-cell contact may further result in production of reactive oxygen species (ROS).

Ren SS, et al. Arch Androl. 2004



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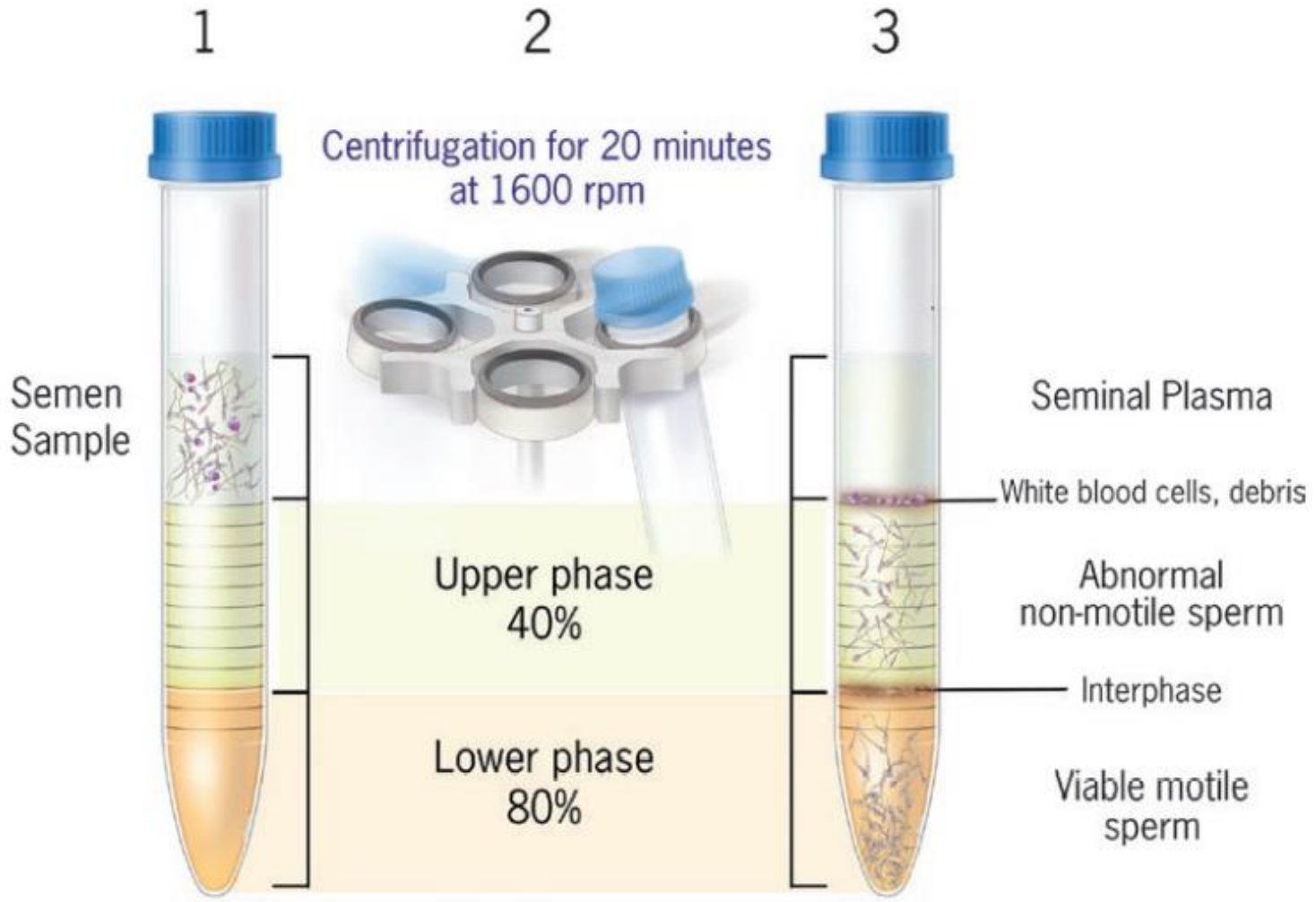
Density Gradient Centrifugation

- It is considered the gold standard technique for sperm preparation. It separates cells based on the density, motility, and centrifugation speed.

- Morphologically normal and abnormal spermatozoa have different densities. Mature morphologically normal sperm are denser (1.10 g/mL) compared to immature and morphologically abnormal sperm (1.06–1.09 g/mL).

- This method allows for the enrichment of mature and motile sperm, and recovery rates of 30–80% can be achieved depending on the initial semen sample and the technical skill of the individual doing the procedure.

- The disadvantages are that the interphases between the layers may take some time; there are reports that sperm prepared by density gradient still have some degree of DNA fragmentation.



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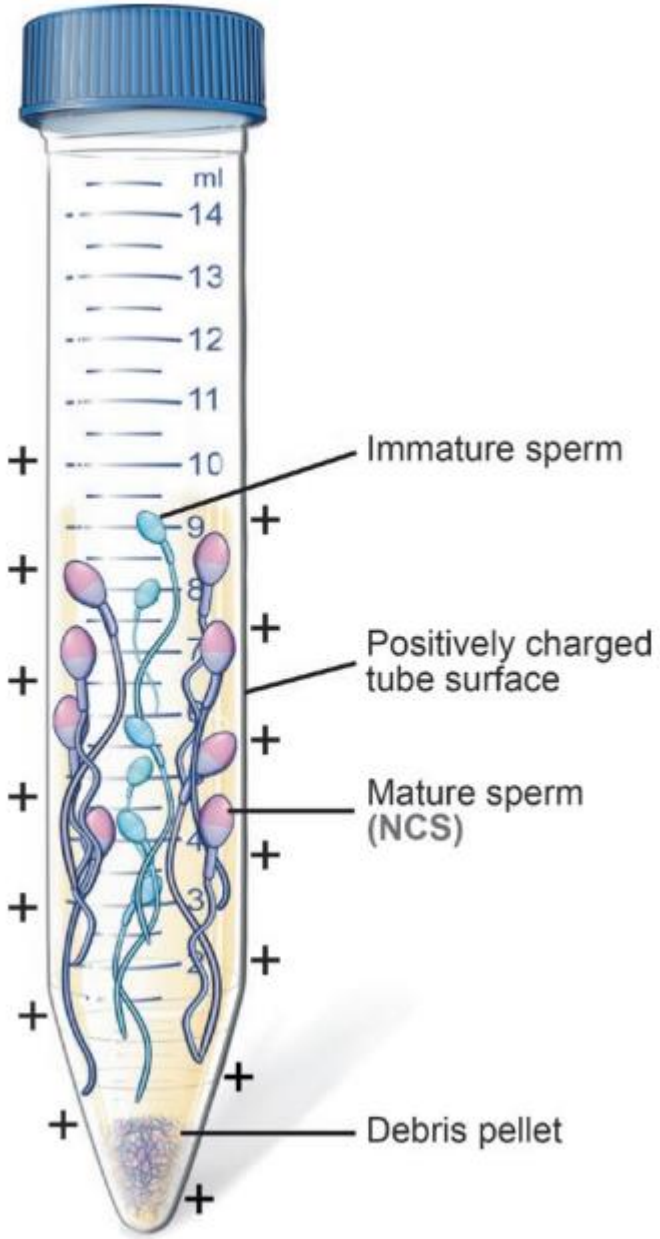
Advanced Sperm Preparation Methods

- Zeta Potential
- Sperm Birefringence
- Motile sperm organelle morphological examination (MSOME)
- Intracytoplasmic morphologically selected sperm injection (IMSI)
- Hyaluronic Acid-Mediated Sperm Selection
- Electrophoretic Sperm Selection
- Microflow Cell
- Annexin V and MACS Separation
- Microfluidic Separation of Sperm

Zeta Potential

- The electrical potential between the sperm membrane that is **negatively charged** and its surrounding is called zeta potential. Negative charge is due to the presence of the epididymal proteins that are present on the sperm membrane surface.

Chan PJ, et al. Fertil Steril. 2000



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- **Markers of apoptosis** were significantly reduced in zeta selected sample.
- Zeta selection results in a significant **reduction** in progressive motility and is **not** very helpful when used in **cryopreserved sperm**.
- Protamine-deficient sperm are eliminated, and sperm with **DNA integrity** are retained resulting in high fertilization rate.
- Negative zeta potential sperm in IVF had a **higher fertilization rate** (**65.79%**) compared with sperm isolated with double density gradient centrifugation.

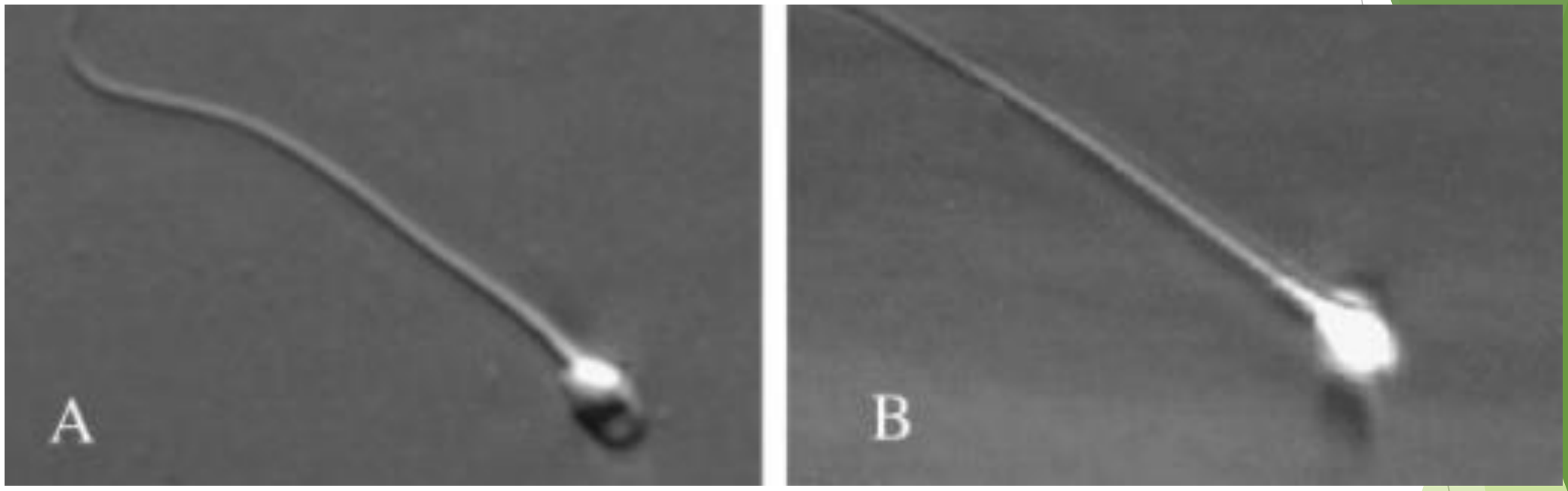
Polak de Fried E, et al. Fertil Steril. 2010

Sperm Birefringence

- **The subacrosomal protein filaments** are longitudinally arranged in mature sperm. Therefore mature sperm nucleus exhibits higher birefringence that can be examined by **polarized light microscopy.**

- This allows the evaluation of birefringence and selection of mature sperm. Acrosome intact sperm with high DNA integrity can be selected from the acrosome-reacted spermatozoa with DNA fragmentation using this technique.

Gianaroli L, et al. Fertil Steril. 2008



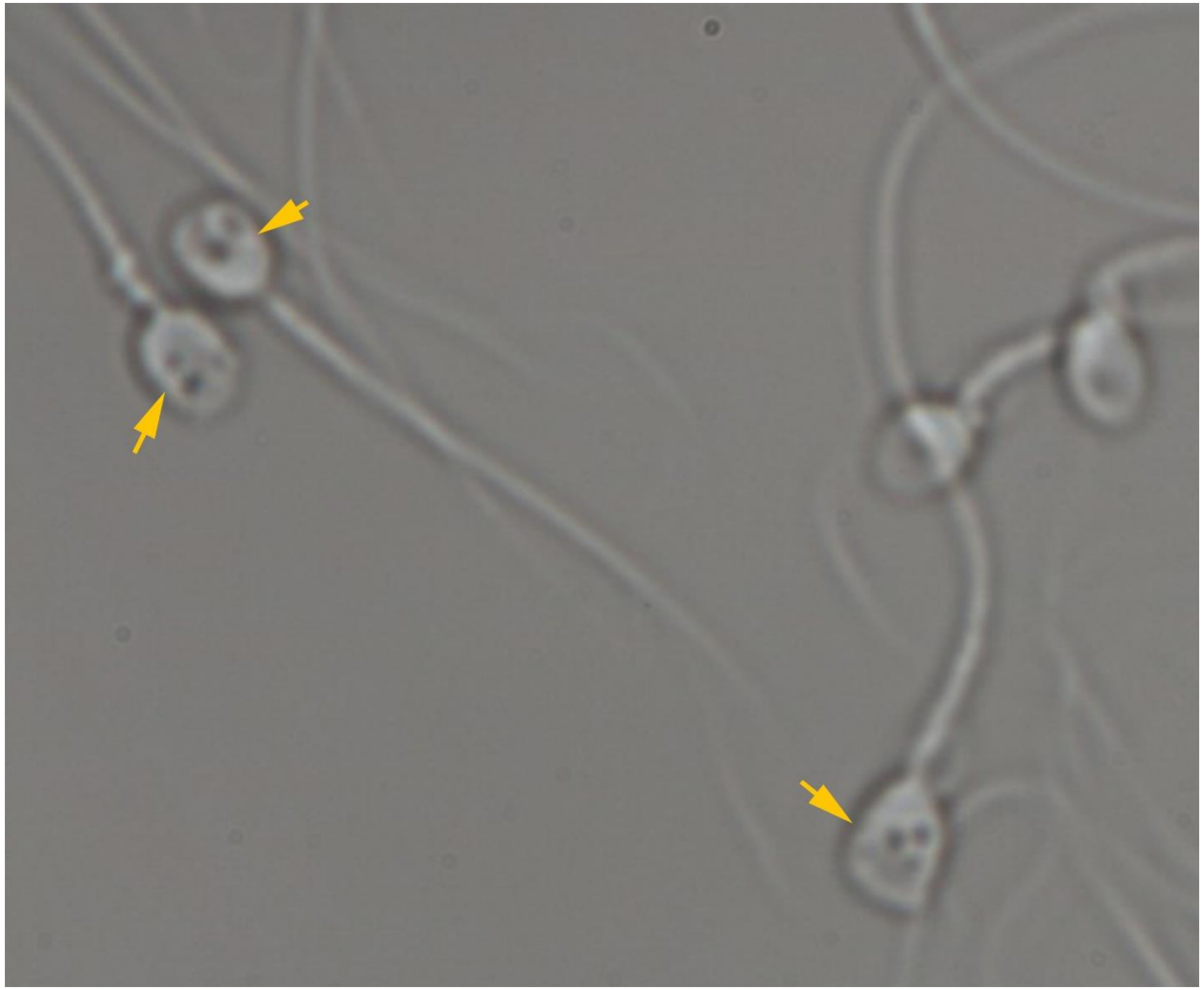
Gianaroli L, et al. Fertil Steril. 2008

Motile sperm organelle morphological examination (MSOME)

- Subtle defects in the sperm morphology in the **acrosome**, **nucleus**, **mitochondria**, **post-acrosomal** lamina, and **neck** can be observed using **real-time** inverted light microscope equipped with **Nomarski** optics enhanced by digital imaging.

- It achieves an ultra-high magnification microscopy (**6300x**) called motile sperm organelle morphological examination (MSOME).

- MSOME was also shown to be positively associated with both **fertilization rate** and **pregnancy outcome**.



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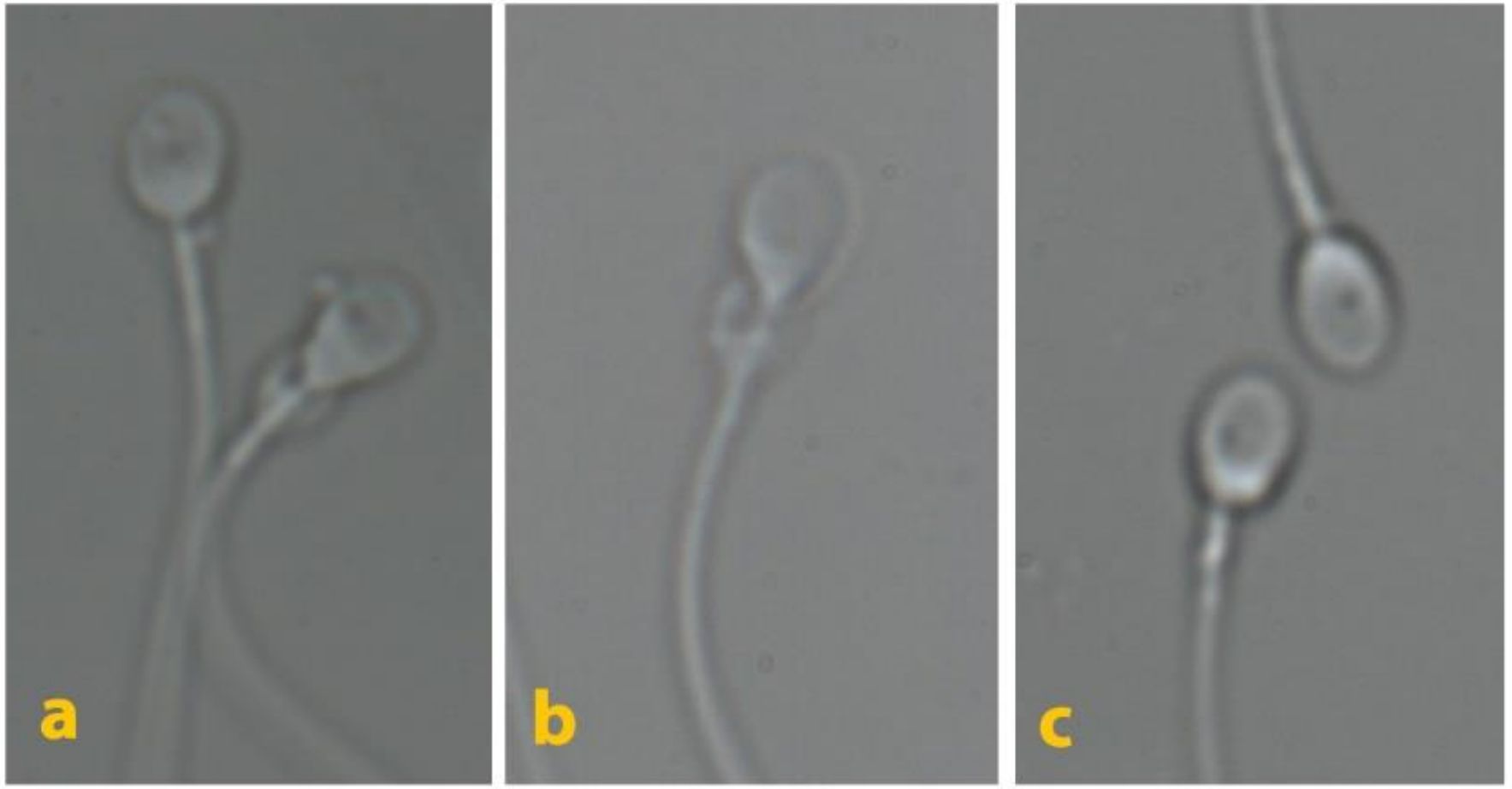
- A correlation was also reported between higher fraction of sperm with **high DNA fragmentation** and presence of **large nuclear vacuoles.**

- The procedure however is **time-consuming**, and the selected sperm may still be exposed to **oxidative stress.**

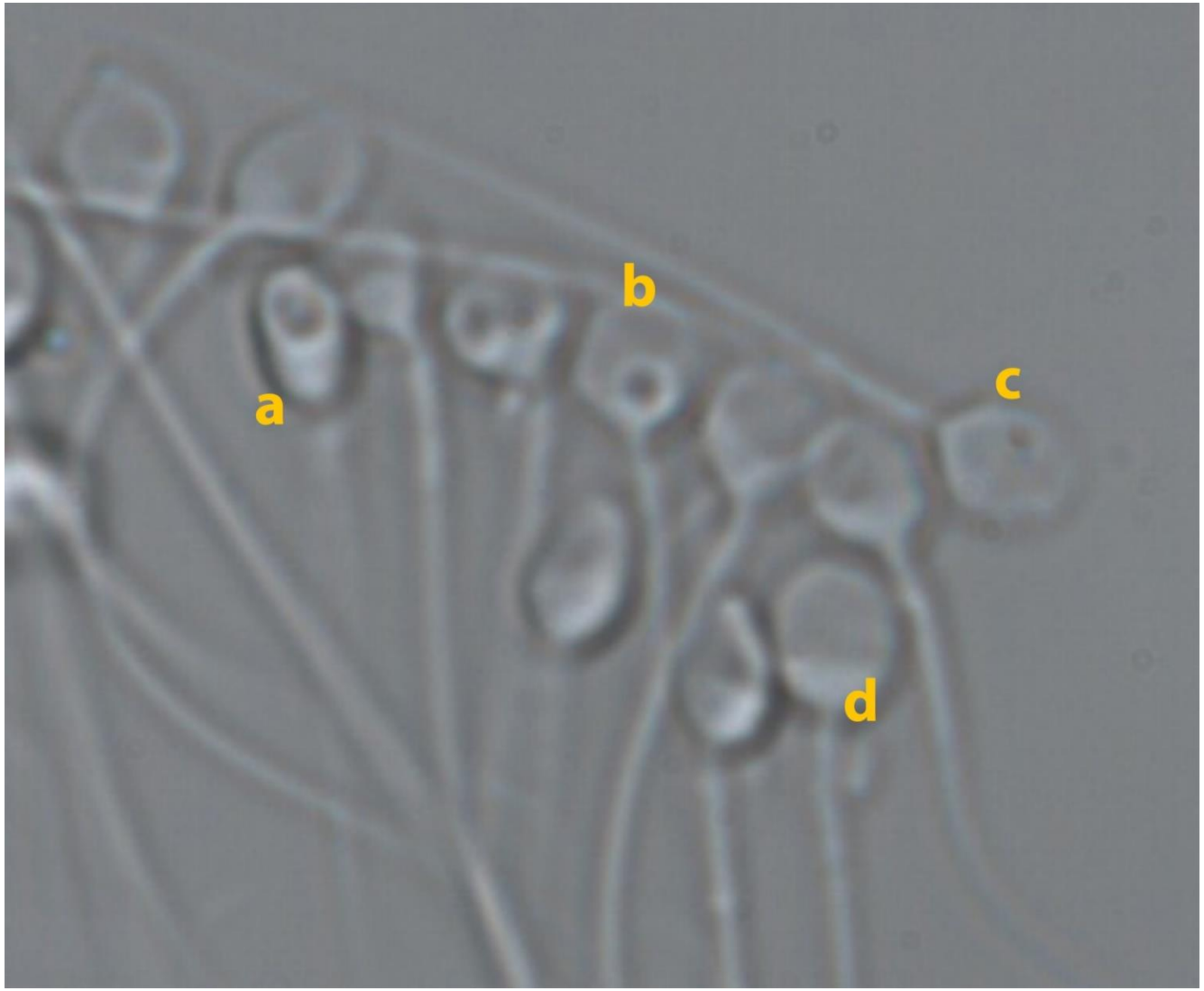
- a recent meta-analysis included 9 randomized controlled trials and 2014 couples (IMSI = 1002; ICSI = 1012) compared regular ICSI for assisted reproduction. The results from this study show lack of evidence that IMSI improves clinical pregnancy rates compared to ICSI.

Bartoov B, et al. J Androl. 2002

Teixeira DM, et al. Cocharane Database System Rev. 2013



Cassuto, 2009



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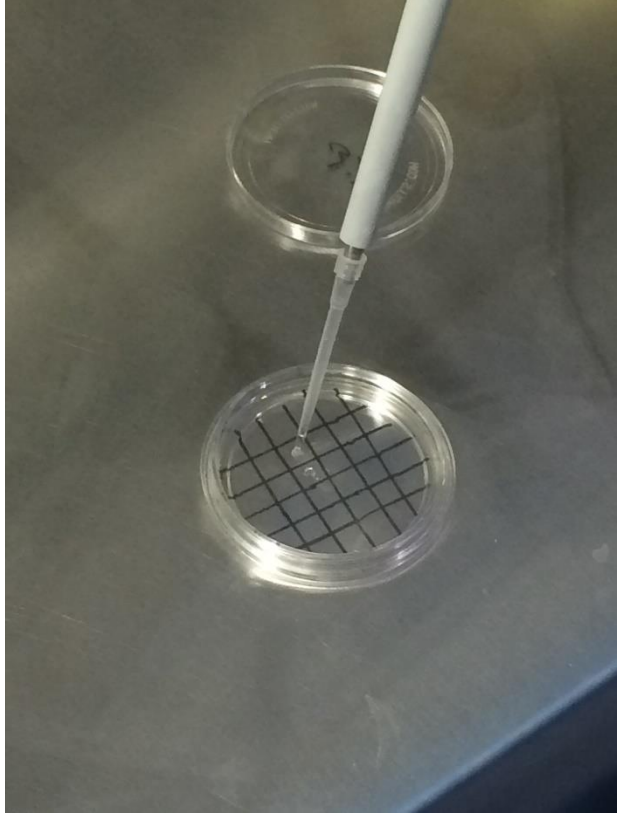

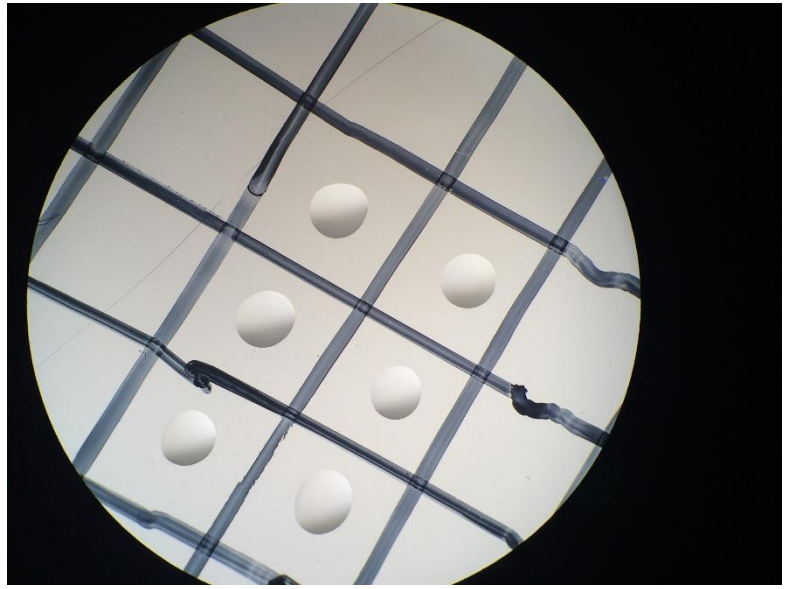

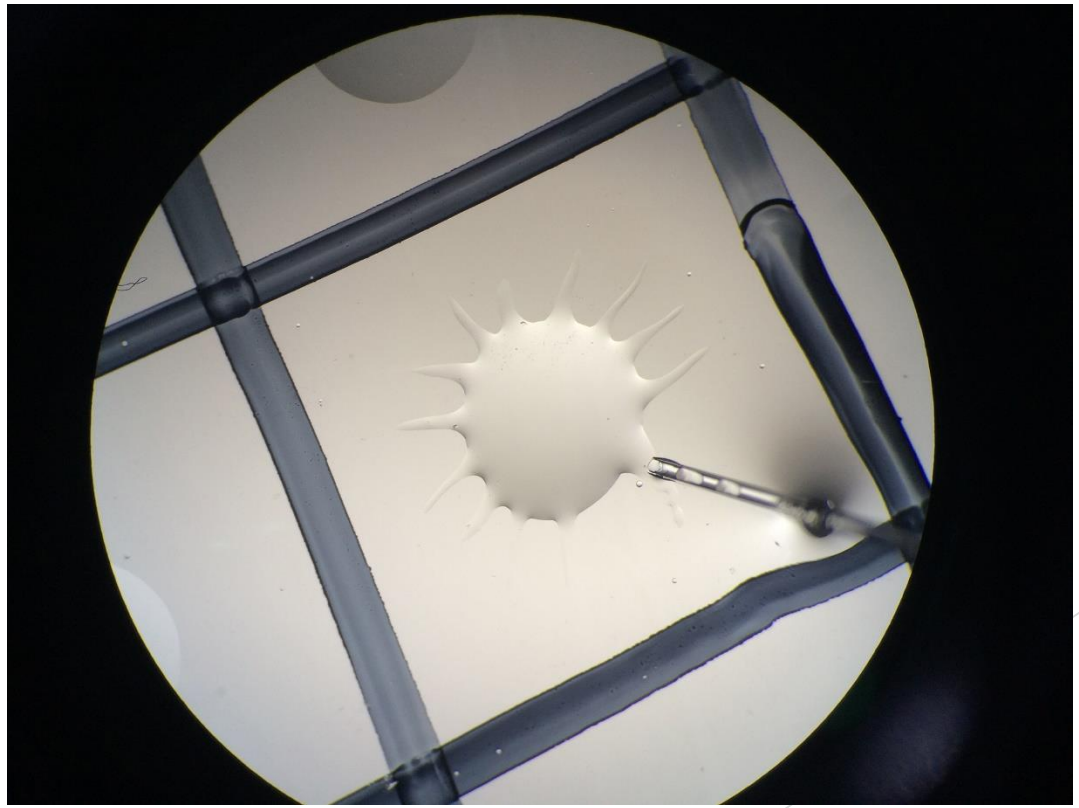

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Hyaluronic Acid-Mediated Sperm Selection

- **Hyaluronic acid receptors** are present on the plasma membrane on **acrosome-intact sperm** and are indicative of **sperm maturity.**
- Sperm can be selected by **physiological intra-cytoplasmic sperm injection (PICSI)** which is a **plastic dish** containing spots of HA attached to its base.
- The frequency of sperm with **chromosomal disomy** is significantly reduced when compared with ejaculated sperm.
- Hyaluronic acid binding also excludes immature sperm with **cytoplasmic extrusion**, presence of sperm with **histones**, and **DNA fragmentation** indicating selection of sperm with **reduced oxidative stress.**

Huszar G, et al. Reprod Biomed Online. 2007

Electrophoretic Sperm Selection

- Sperm can be separated based on their **size** and **charge** on their surface by electrophoretic separation.

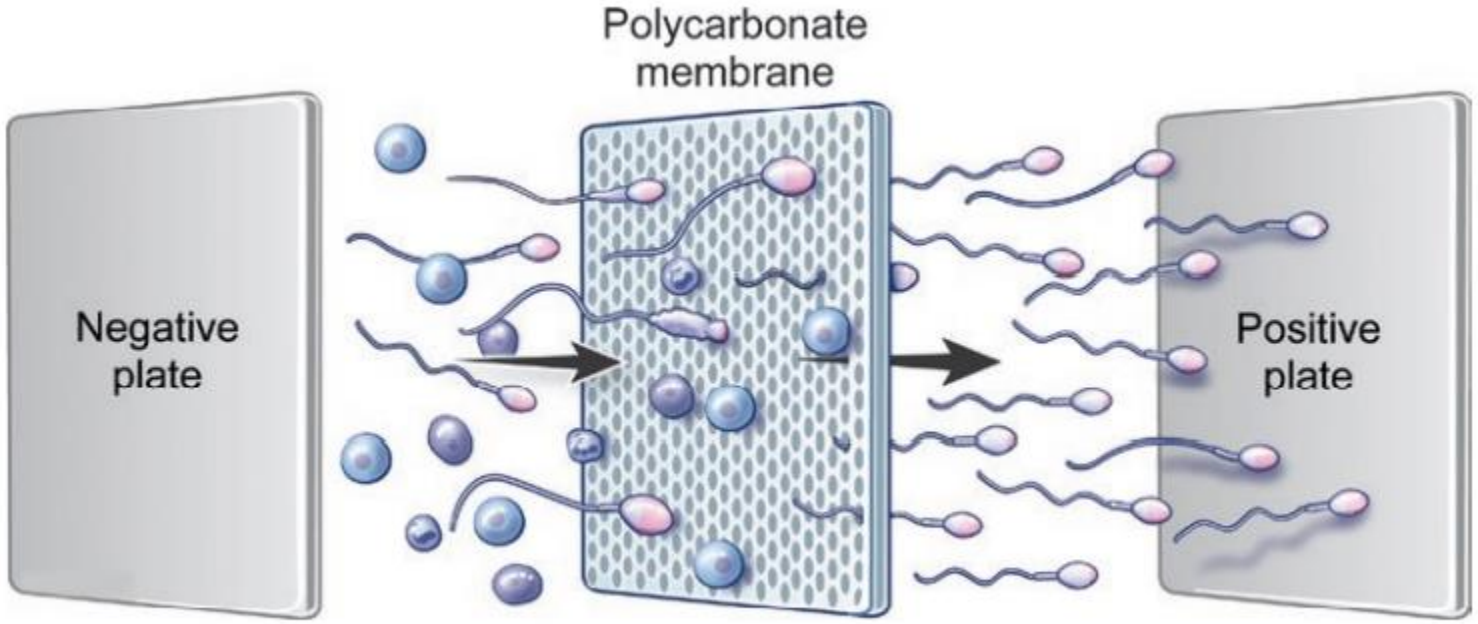
- Normally differentiated sperm are charged **negatively**. The resulting population shows a **low** incidence of **DNA damage**. It compares favorably with the density gradient separation technique in purity, **absence of ROS**, and **superior viability** and **morphology** of the isolated spermatozoa. Motility has been reported to be affected by electrophoresis.

Ainsworth C,et al. Hum Reprod. 2005

Microflow Cell

- A microflow cell consists of an **outer chambers** connected with a platinum-coated titanium electrodes, and the **inner chamber** is divided into two compartments – the inoculation **(loading) chamber** and the **collection chamber**.

- A **polycarbonate membrane** 5 μm thick separates the two compartments. The membrane filters out the good-quality sperm from the contaminating cells such as the leukocytes and germ cells



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- The membrane **filters** out the good-quality sperm from the contaminating cells such as the **leukocytes** and **germ cells**. A 400 µL semen sample is loaded in the inoculation chamber and the buffer in both loading and collecting chambers, and sample is equilibrated for 5 min.

- A constant current of 75 mAmps is applied with a variable voltage of 18–21 mV. The **highly motile good quality spermatozoa** are sorted out and are ready to be used in ART.

Annexin V and MACS Separation

- **Phosphatidylserine** is a phospholipid that is present on the inner leaflet of the plasma membrane. It moves to the outer surface when the membrane is damaged. Thus externalization of the phosphatidylserine residue is a **marker** of **apoptosis**.

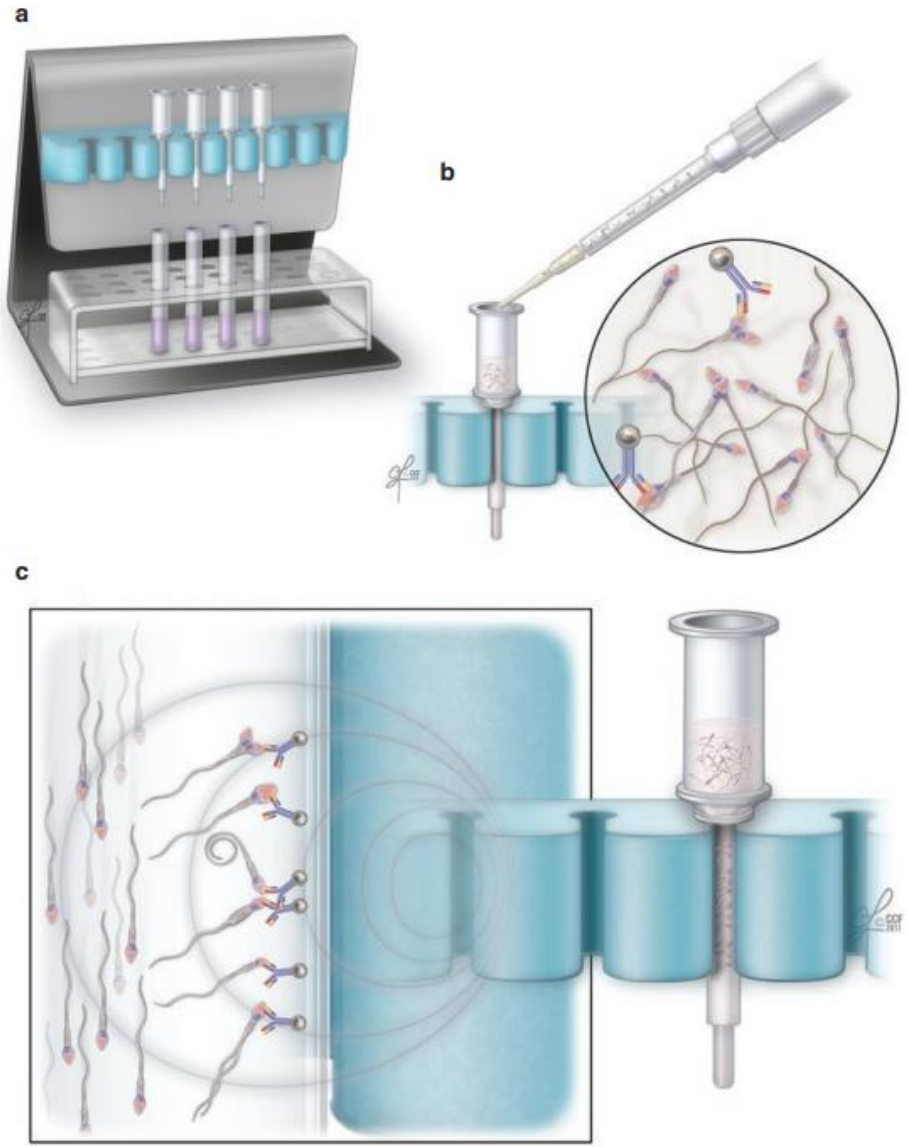
- Reactive oxygen species (**ROS**) not only affects nuclear and mitochondrial DNA but also is involved in the activation of **apoptosis signaling cascade** parts.

- **Annexin V** is a phospholipid-binding protein. It has a strong affinity for phosphatidylserine residue. Therefore annexin V is used to **label sperm** that have a **compromised membrane integrity** and are less able to fertilize the egg.

- Magnetic activated cell sorting (MACS) uses a colloidal super-paramagnetic microbeads conjugated with annexin V antibodies. A strong magnetic field is employed, and the sperm that are **non-apoptotic** pass through the magnetic field, whereas those that are **apoptotic** are tagged and retained in the magnetic field.

- While density gradient removes immature sperm cells, debris, and leukocytes, the annexin V MACS removes already **damaged sperm** with altered membranes, activated apoptosis signaling, and DNA fragmentation.

Grunewald S, et al. Cell Tissue Bank. 2001



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Microfluidic Separation of Sperm

- This is the **latest** in sperm selection technologies. Microfluidic devices use **microchannels** made from polydimethylsiloxane (**PDMS**) silicon polymers that are nontoxic and transparent.

- **Lab-on-chip** approaches have been used to select sperm based on **motility**, **chemotaxis**, and **electrophoresis**.

Tasoglu S, et al. Small. 2013

Sperm can be selected using;

- (1) passively driven microfluidic device
- (2) chemoattractant microfluidic device
- (3) chemotaxis device
- (4) microfluidic fertilization device
- (5) macro-microfluidic sperm sorter
- (6) Zech selector
- (7) circular microfluidic device
- (8) microgroove and channel device
- (9) boundary-following behavior-based passive microfluidic device

- A **simple** clinically applicable lab-on-a-chip method was reported for sperm selection based on progressive motility in **500** parallel microchannels. In this one-step procedure, 1 mL of semen could be processed under 20 min resulting in over **80% improvement** in selected sperm **DNA integrity**.

Nosrati R, et al. Lab Chip. 2014

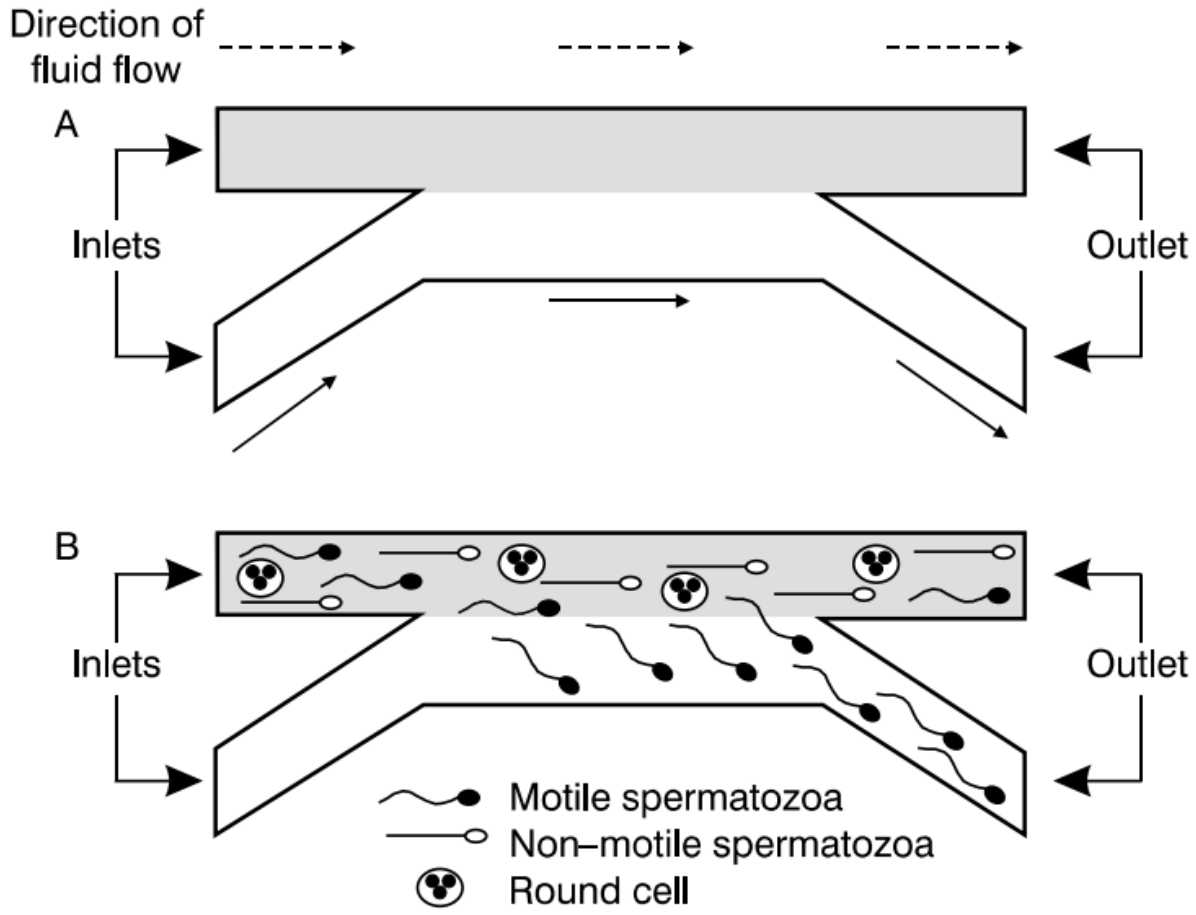
- The **major advantage** of microfluidic devices over conventional selection techniques is the ability to work with **small sperm sample volume**, **the short processing times**, and the ability to **manipulate single cells** in a **noninvasive** manner.

- The **yield** of the selected sperm by microfluidics is about **41%** and comparable to recovery rates of currently used methods.

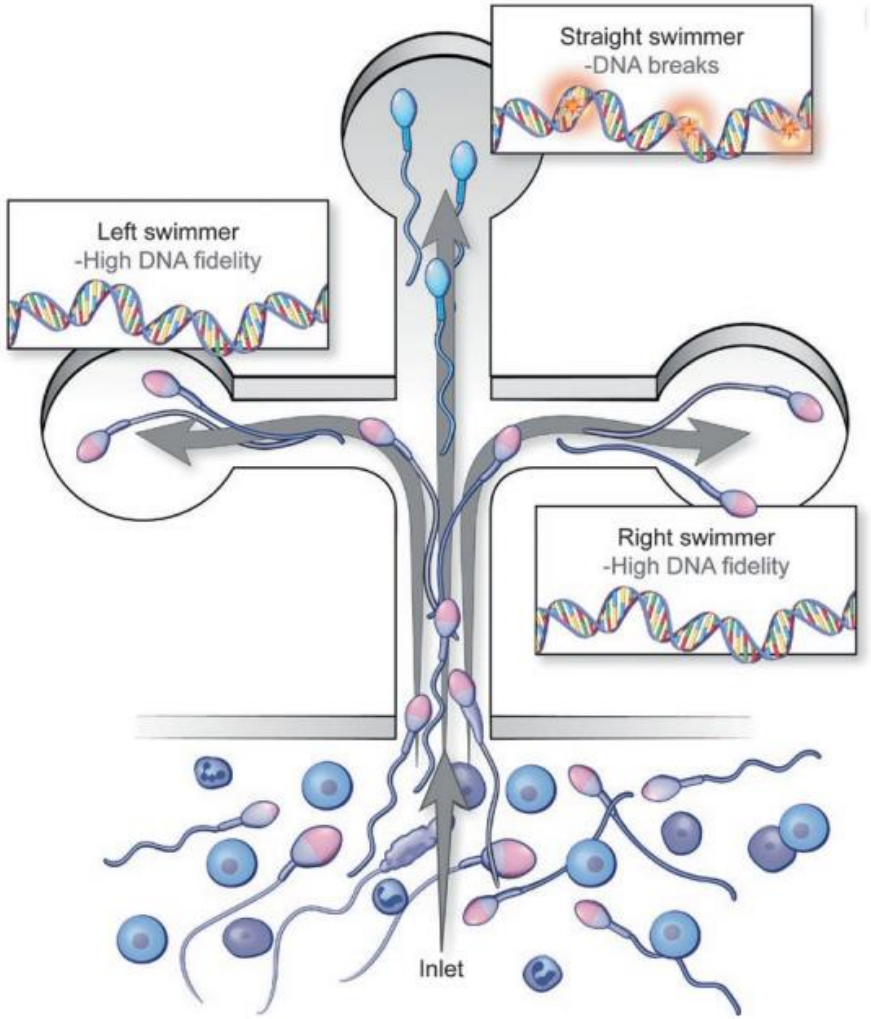
- Another advantage is the **one-step process** that **eliminates centrifugation** and the exposure to reactive oxygen species and thereby preserves the DNA integrity.

- Fertilization of ova with preselected spermatozoa of superior quality by microfluidic technique was accomplished using a **robotic-assisted platform** and **IVF on a chip**.

Tsai YF, et al. Urol Sci. 2016



Schuster et al, Reprod Biomed Online 2003



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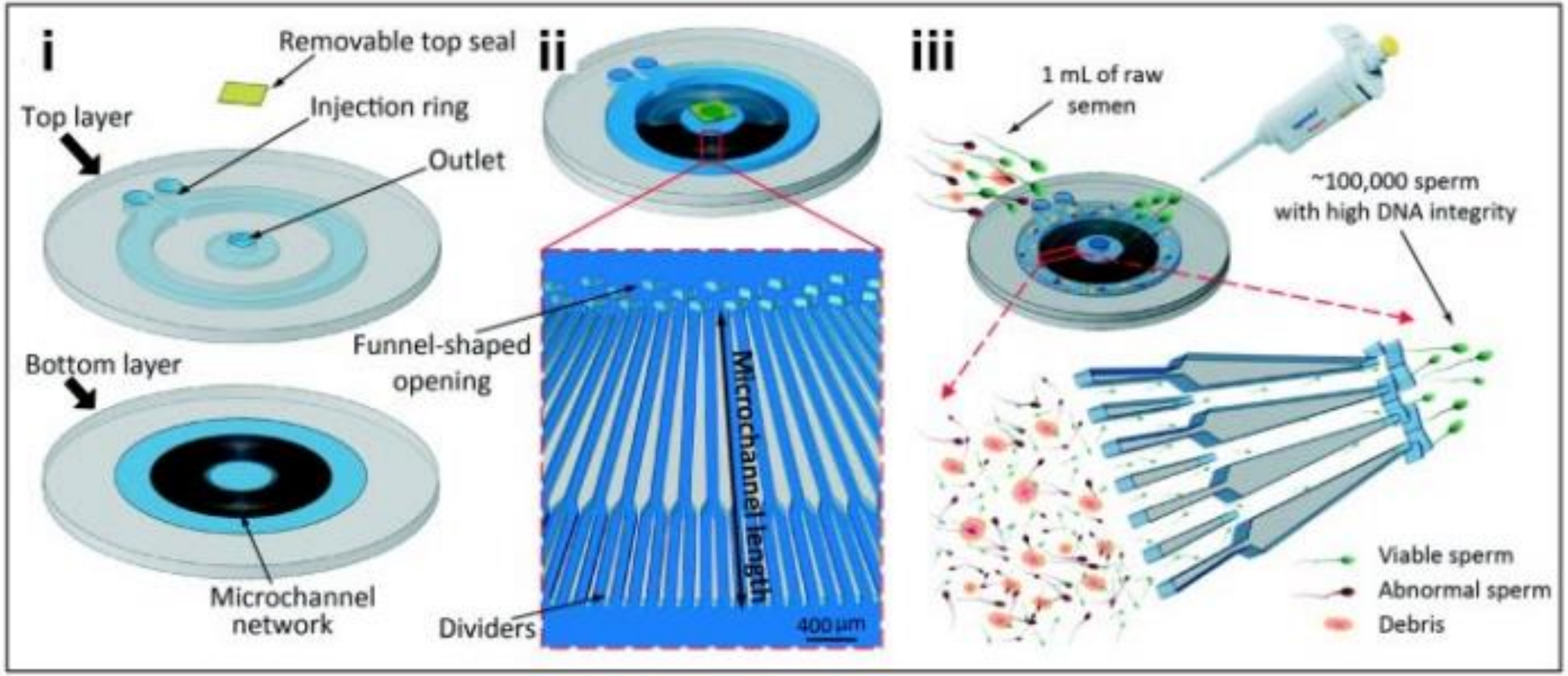
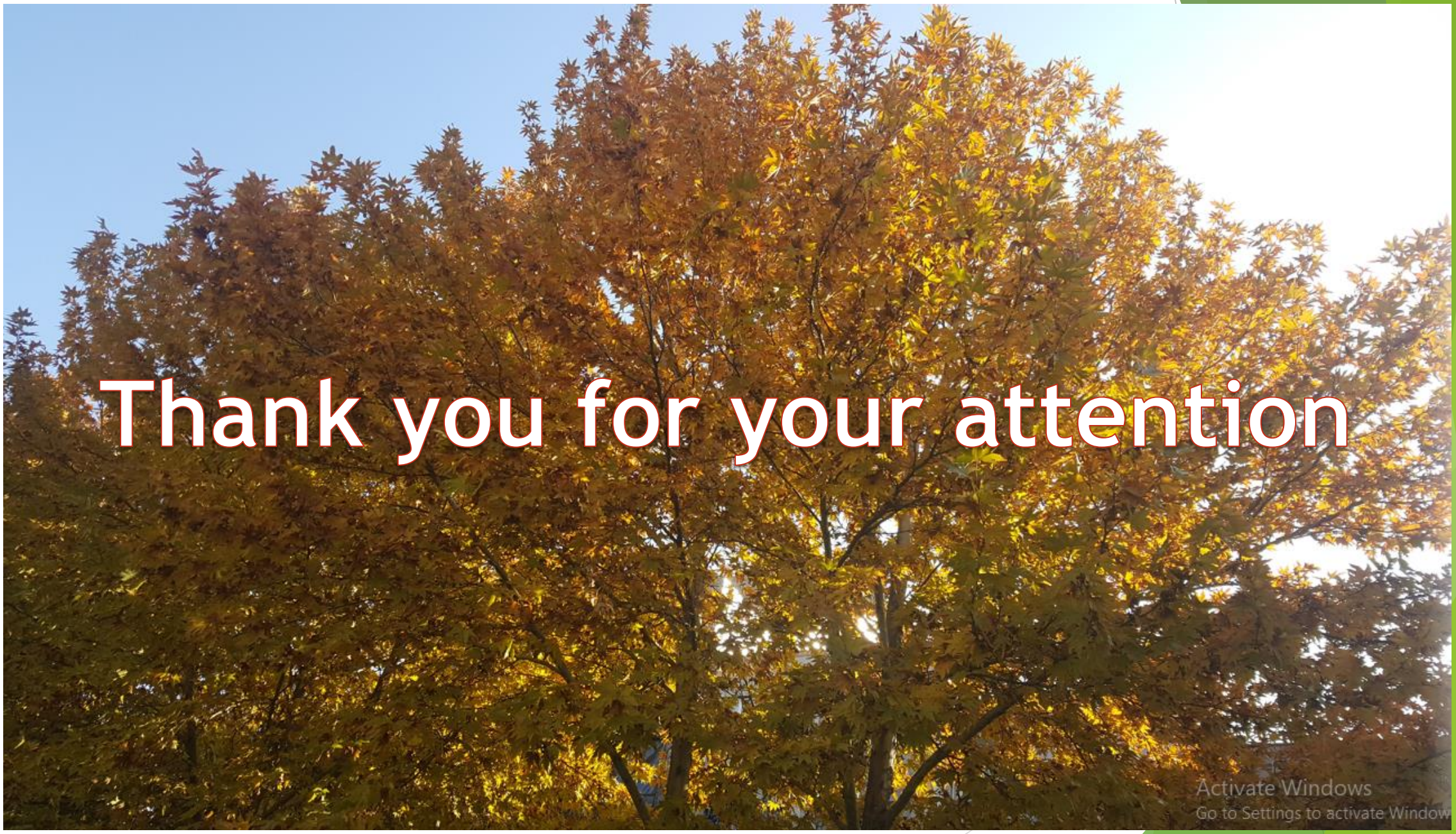


Nosrati et al, Lab Chip 2014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