Primer on sperm preparation for Human ART

Introduction

As explained in Cytoswim's earlier document Why Selecting Spermatozoa by Motility is the Most Natural Approach [1], it is clear that in Nature the sperm that fertilize oocytes in vivo come from populations that are largely self-selected within the female reproductive tract based on their inherent motility. This was why pioneers of fertility treatment using IUI, and in the early days of IVF, prepared sperm from semen by swim-up rather than simple repeated sperm washing by centrifugation. But during the 1980s many alternative methods were elaborated to try and select "the best" sperm, or more sperm, or as many sperm as possible from poor ejaculates, or just doing it quicker and easier.

This Primer discusses the theory and limitations of the various methods that are available for sperm preparation for clinical purposes in the andrology or ART laboratory [see ref.2 for more details] .

Methodological Approaches

Direct swim-up from semen (DSUS)

Pros:

·The earliest, simplest method for sperm preparation; no specialized devices or products needed.

· Separates the most motile sperm, which have generally better morphology.

Cons:

·Yield depends on % motile and progression quality.

·Takes longer, with lower yield, for less motile specimens.

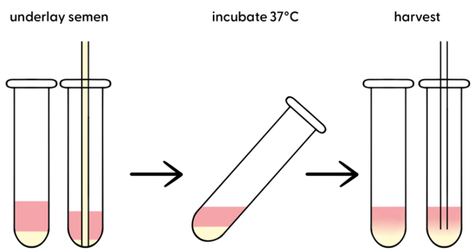
Method:

·Either layer culture medium over liquefied semen or semen under the medium in a round bottom tube; the latter gives the cleaner preparation.

·Optimize the semen-medium interface area and the semen:medium volume ratio to maximize the yield.

·Incubate at 37°C for 20–60 min then harvest the upper fraction of the medium layer.

· Typically use 1 centrifugal wash of the selected sperm (max 500 g × 15 min).



Swim-up from washed pellet (SUWP)

Promoted in the early days of IVF (1980s) when IVF was used largely for tubal factor infertility.

Pros:

·Separates the most motile sperm, which have generally better morphology.

Cons:

·Risks significant ROS damage to the sperm within the pellet.

·Yield depends on % motile and progression quality.

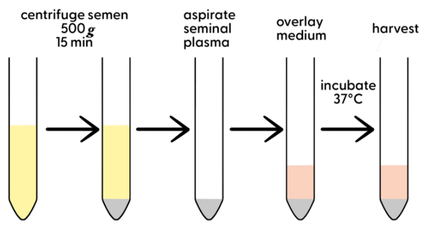
·Takes longer, with lower yield, for less motile specimens.

Method:

·Add medium to liquefied semen and centrifuge; discard the supernatant and overlay the pellet with culture medium. Can 'loosen' pellet slightly before over-layering medium.

·Incubate at 37°C for 20–30 min, then harvest the upper fraction of the medium layer.

· Typically use 1 centrifugal wash of the selected sperm (max 500 g × 15 min).



Trans-membrane migration (TMM)

First described for research on sperm selection using a nickel mesh, and in the early 1980s for studying the effects of drugs on sperm motility using a Nuclepore™ membrane.

Pros:

· Separates the most motile sperm, which have generally better morphology.

· A membrane separates the medium layer from the liquefied semen to prevent mixing.

Cons:

·Requires specialized, often costly, devices.

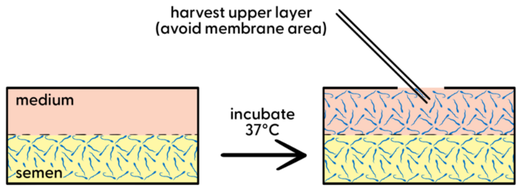
·Yield depends on % motile and progression quality.

·Takes longer, with lower yield, for less motile specimens.

Method:

·Incubate at 37°C for 15–60 min; harvest the medium layer.

· The basis of the ZyMot® Multi devices.



Adhesion/filtration and Zeta potential methods

Based on the negative electric charge on the surface of mature live sperm (due to sialoglycoproteins that accumulate on the plasma membrane late in sperm maturation).

Removes the more positively charged ('stickier') dead sperm that bind to the glass or gel filtration beads, while the motile, mature sperm pass through the column.

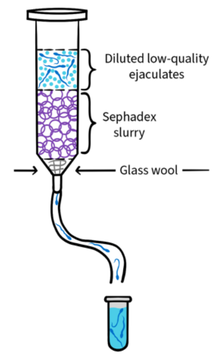
Pros: Shorter processing times.

Cons: Requires specialized products.

Method:

· Early methods based on the 'sticking-to-glass' phenomenon used glass beads and glass wool (risk of glass fragments). Later methods included Sephadex® beads (negatively charged dextran-based gel filtration product).

· Zeta potential is now being explored as a possible marker of sperm functional competence.



Electrophoresis-based methods

Based on the negative electric charge on the surface of mature live sperm (due to sialoglycoproteins that accumulate on the plasmalemma late in sperm maturation).

Pros:

·Separates the more mature motile sperm.

·Shorter processing times.

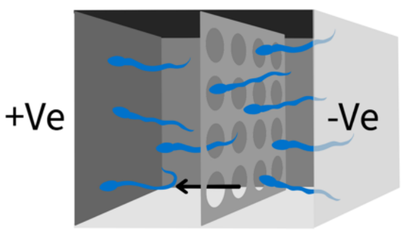
Cons:

·Requires specialized equipment and consumables.

·No medical device products commercially available yet.

Method:

·The Felix™ system combines the migration of mature sperm towards the anode with passage through a membrane filter.



Density gradient centrifugation (DGC)

Based on the difference in density between a mature human sperm (>1.12 g/ml) and the less dense immature sperm.

Pros:

· Isopycnic separation of the most mature sperm, which have generally excellent motility and better morphology.

· Does not depend on sperm motility so gives maximum yield in a fixed processing time

· Colloidal silica avoids the problems with high viscosity of other dense media; Percoll® was used in the early days, but silane-coated silica-based products (e.g. PureSperm®, Isolate) since 1997.

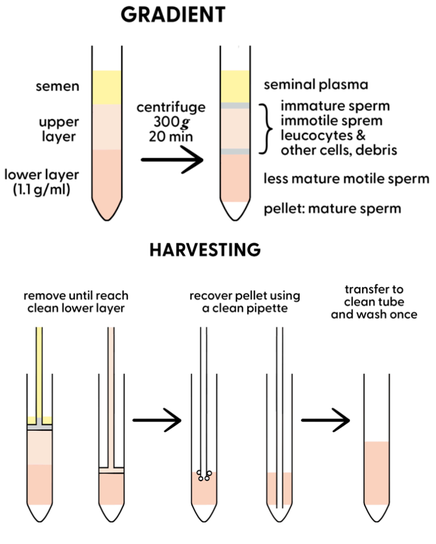
Cons:

·If DGC products not containing EDTA are used there is a significant risk of ROS damage to the sperm during centrifugation and pelleting.

Method:

·Typically 2 layers: a lower colloid layer with a density of 1.1 g/ml and an upper colloid layer around 1.05 g/ml (semen is ~1.03 g/ml).

· Centrifuge at 300 g × 20 min then recover the pellet cleanly (avoiding seminal plasma contamination is vital); resuspend in culture medium and wash once (max 500 g × 15 min); a further swim-up step is not needed.



Magnetically-activated cell sorting (MACS beads)

MACS® MicroBeads are nano-scale colloidal super-paramagnetic beads with annexin-V conjugated onto their surface so they bind to externalized phosphatidylserine residues on the surface of apoptotic sperm.

Pros: Non-apoptotic sperm have lower levels of fragmented DNA.

Cons:

·Motile sperm are initially selected using either DSUS or DGC before the MACS procedure, which is followed by a centrifugation step to concentrate the final sperm prep.

· Time consuming and expensive.

· Sole supplier of the specialized reagents.

Method:

·Under a strong magnetic field the sperm with beads bound to them are removed from the cell suspension.

EHyaluronan binding

Based on the ability of fully mature sperm to bind to hyaluronan, these sperm usually have better quality DNA.

Pros: Identifies fully mature sperm.

Cons:

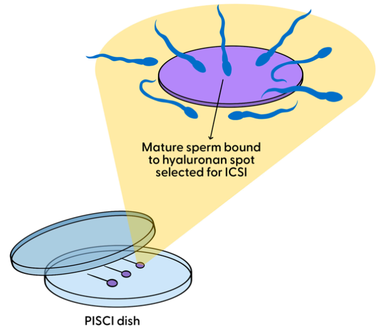
·Sperm are typically prepared by DGC first.

·Specialized, costly devices.

·Performance still controversial; questionable clinical value.

Method:

·Can be employed as either a diagnostic test (HBA® Slide) or for selecting sperm to be used for ICSI (PICSI® dish).



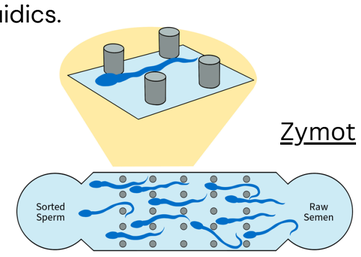
Microfluidics-based methods

Microfluidics is the study and manipulation of fluid flow at the sub-millimetre scale, often through micro-channels (often described as 'lab-on-a-chip').

Pros: Devices attempt to replicate the physico-chemical processes that operate within the female reproductive tract, and employ large scale parallelism to achieve high yields.

Cons: There are many reports of such devices in the research literature, but currently no commercial devices for clinical application.

Method: Most commercial so-called "microfluidic" methods do not actually employ active microfluidics, i.e. flow, but are based on guided migration (see below), although this approach is sometimes referred to as passive microfluidics.



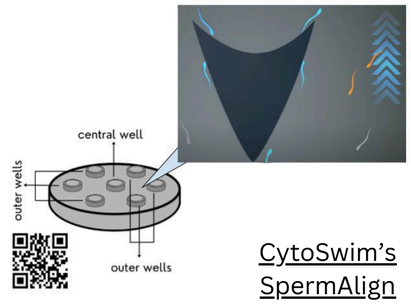
Zymot

Guided migration-based methods

Devices attempt to replicate the physico-chemical processes that operate within the female reproductive tract, by creating micro scale boundaries, channels or rows of columns that direct sperm to swim through them. Large scale parallelism is used to achieve higher yields.

·While there are many devices of this type described in the research literature, there are few commercial devices for clinical application as yet.

· Commercial clinical products include the ZyMot® ICSI device and CytoSwim's SpermAlign device.



CytoSwim's SpermAlign

Discussion

Because centrifuging whole semen can lead to the generation of damaging levels of reactive oxygen species that can adversely affect sperm function, and even sperm DNA, the direct swim-up of spermatozoa (DSUS) from liquefied semen is by far the safest approach. However, from the mid-1980s density gradient centrifugation (DGC) methods became more common in the vast majority of IVF labs due to their consistently higher yields of motile spermatozoa and shorter processing times.

Provided that properly formulated products (i.e. containing EDTA to protect against potential ROS-induced damage arising from possible heavy metal contamination of the colloid), and extremely careful pellet harvesting techniques are employed, DGC is a very safe procedure for selecting the most dense – and hence the most mature – sperm, but many labs remain unaware of these best practices.

But the technical complexity of optimized DGC procedures, combined with considerations such as the growing shortage of competent clinical embryologists and the desire to make ART procedures both cheaper and more sustainable (less plastic, less culture media), make simpler methods increasingly attractive.

With the advent of simple stabilized swim-up devices (e.g. ZyMot® Multi devices) and sperm migration passive microfluidic devices (e.g. ZyMot® ICSI and CytoSwim SpermAlign devices), as well as active microfluidics-based devices, all of which achieve increased yields of selected functional sperm with lower levels of DNA damage, there has been a wide resurgence in interest in selecting spermatozoa for use in assisted conception procedures using Nature's way of doing this in vivo: via their intrinsic motility. Current issues mainly concern cost-effective large scale manufacture.

Conclusion

Selecting human sperm based on their innate motility, ideally augmented via physical constraints within guided migration devices, is a clear example of biomimicry, and hence is the most natural approach to sperm preparation for use in assisted conception. Yields sufficient for IVF are easily achievable, but obtaining sufficient sperm for IUI treatment can be problematic.

References

[1] why-selecting-spermatozoa-by-motility-is-the-most-natural-approach

[2] Mortimer D, Björndahl L, Barratt CLR, Castilla JA, Menkveld R, Kvist U, Alvarez JG, Haugen TB. A Practical Guide to Basic Laboratory Andrology, 2nd ed. Cambridge University Press, Cambridge, UK, 2022.