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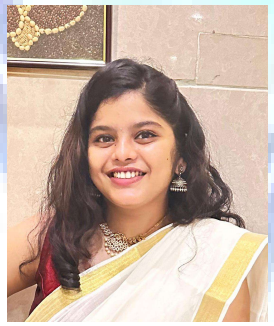
TOPIC OF DISCUSSION

"Sperm Selection in ART : Are We Stuck in Tradition or Open to Innovation ?"

CHAT DISCUSSIONS COMPILED BY



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SUMMARY OF SURVEY RESULTS

"What Methods of Sperm Preparation do you prefer for ICSI For Normal Semen Parameter (WHO 2021) ?"

By Team Ihera

INTRODUCTION

Sperm selection is a crucial step in assisted reproductive technologies (ART), significantly influencing outcomes in procedures like intrauterine insemination (IUI) and in vitro fertilization (IVF). Given the advancements in reproductive science, various sperm selection methods have been developed to optimize sperm quality and enhance the chances of successful fertilization and pregnancy.

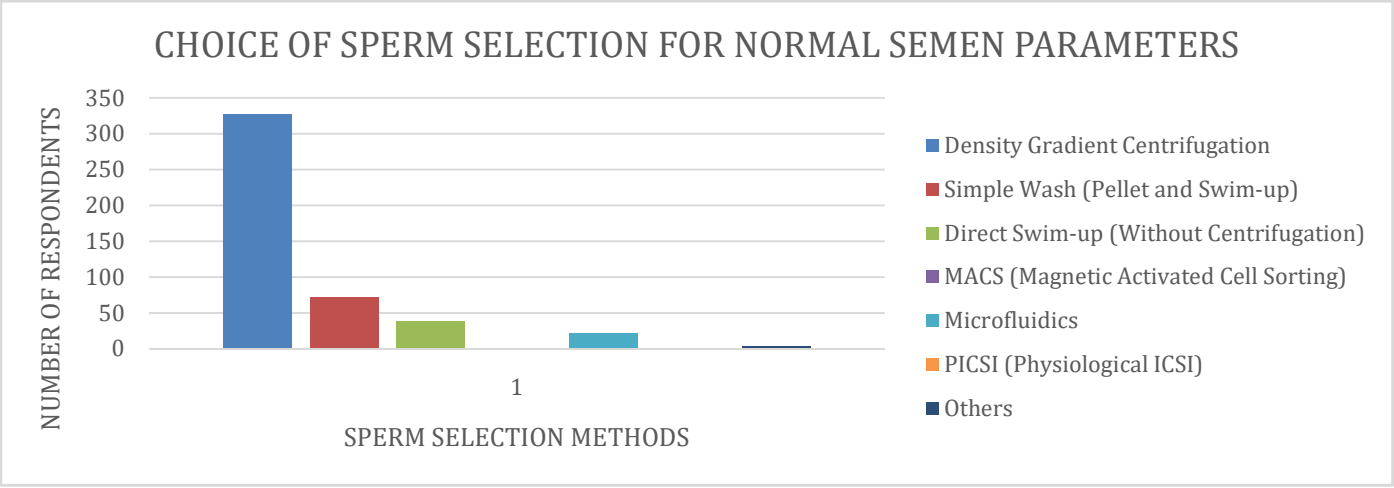
This survey aims to provide a comprehensive overview of the preferences and practices among embryologists worldwide regarding sperm selection methods, specifically focusing on cases with normal semen parameters as defined by the World Health Organization (WHO) 2021 standards.

In this survey, we explore the adoption and frequency of different sperm selection techniques, including Density Gradient Centrifugation, Simple Wash methods (such as Pellet and Swim-up), Direct Swim-up without Centrifugation, Magnetic Activated Cell Sorting (MACS), Microfluidics, Physiological ICSI (PICSI), and other emerging or less common methods. By analysing these preferences, the survey seeks to shed light on current global trends, highlight the advantages and limitations of each technique, and ultimately contribute to a better understanding of best practices in sperm selection for ART.

The data presented herein reflects the diverse approaches employed by embryologists to address the nuances of sperm selection and aims to foster discussions on optimizing techniques for improved ART outcomes.

Poll Results: Strong Preference for Density Gradient Centrifugation

A poll was conducted among embryologists and ART professionals to assess their preferred methods of sperm selection for normal semen samples based on WHO 2021 guidelines.



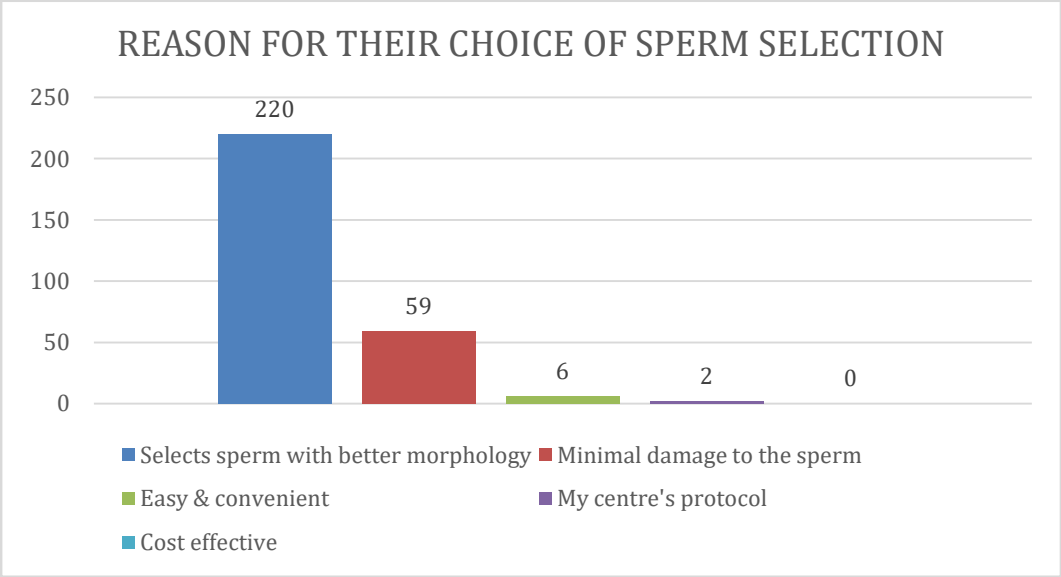
Density Gradient Centrifugation	327	70.3%
Simple Wash (Pellet and Swim-up)	72	15.4%
Direct Swim-up (Without Centrifugation)	39	8.38%
MACS (Magnetic Activated Cell Sorting)	0	-----
Microfluidics	22	4.73%
PICSI (Physiological ICSI)	1	< 1 %
Others	4	< 1 %

Key Insights

- A significant majority (70.32 %) of embryologists prioritize the use of **Density Gradient Centrifugation**, citing its reliability and effectiveness in isolating motile, morphologically normal sperm. This preference reflects the method's widespread use in ART, where sperm quality is crucial for successful outcomes.
- **Simple Wash (Pellet and Swim-up)** was the second most preferred method, chosen by 15.48 % of respondents. **Direct Swim up (without centrifugation)** was the third most preferred method chosen by 8.73% of the respondents. While simpler and less costly than density gradient centrifugation, it may not be as effective in selecting the best quality sperm as Density Gradient, which could explain its lower preference.
- Although **Microfluidics** is considered a cutting-edge technology that mimics natural sperm selection processes, it remains less popular, chosen by only 5% of respondents. This may be due to its relatively recent introduction and higher costs compared to more established methods.

Poll Results: Why Do You Prefer This Method of Sperm Selection?

The poll was conducted to understand the reasoning behind embryologists' preference for specific sperm selection methods. Participants were asked to select their primary reason for choosing their method. The results are as follows:



- **Selects Sperm with Better Morphology**: This was the most popular reason, with the majority (76.65%) of participants indicating that they prefer methods that help select sperm with

better morphology. This reflects the importance embryologists place on sperm shape and structure, which are critical factors for successful fertilization and embryo development

- **Minimal Damage for Sperm**: The second most favoured reason 20.55 % (59 votes) was the minimization of sperm damage. Methods that are less invasive or cause less mechanical stress on sperm were seen as beneficial for preserving sperm integrity, which is essential in ART procedures like ICSI.

- **Easy and Convenient**:

A small group of participants (2.09% - 6 votes) chose methods based on their ease of use and convenience. This may indicate that for some, practical aspects like time efficiency and simplicity are key considerations.

- **My Centre Protocol**:

Only 2 participants stated that their preference is guided by the specific protocol established by their fertility centre, reflecting that in some cases, institutional guidelines dictate method choice.

- **Cost Effective**: 0 respondents

Interestingly, no one cited cost-effectiveness as a reason for their preferred method, indicating that sperm quality and outcomes are prioritized over financial considerations in the selection process.

Key Insights

- **Focus on Morphology:** The overwhelming preference for methods that select sperm with better morphology highlights the crucial role morphology plays in ART success. This suggests that many embryologists believe morphological quality is directly linked to fertilization rates and embryo health.

- **Sperm Integrity Matters:** The second most popular choice, minimizing sperm damage, points to concerns about mechanical or oxidative stress during the selection process, indicating a preference for techniques that are gentle on sperm.

Practical Considerations: While ease of use and convenience matter for a small group, they are less of a driving factor compared to quality-related considerations.

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DISCUSSION SUMMARY

SPERM SELECTION IN ART: ARE WE STUCK IN TRADITION OR OPEN TO INNOVATION?

By Gauthami Bitla

Survey Reveals Classical swim-up and Density Gradient for sperm preparation for ICSI is widely used, whereas depending on the individual organization, other newly emerging methods are used, among Group Members Despite Mixed Evidence.

Poll Result:

Our recent poll revealed that the Density Gradient (DG) method is used by 70.32% of participants. This raises an important question: Is it genuinely the best method, or are we simply adhering to tradition? Meanwhile, Microfluidics (MF), a newer technology, is only employed by 5% of respondents. Is cost the primary barrier to adoption, leading us to prioritize practicality over quality?

Furthermore, do these emerging methods provide better outcomes, or do established techniques still dominate in optimizing Assisted Reproductive Technology (ART) results?

Introduction

A recent online discussion among embryologists nationwide focused on various methods for processing samples for IUI, IVF, and ICSI. Sperm preparation is a crucial first step in ART procedures, significantly influencing the chances of successful pregnancies. The session included a poll followed by a group discussion. Below are the methods reviewed, along with their protocols, advantages, and disadvantages.

METHODS FOR SPERM PREPARATIONS:

1. CLASSICAL SWIM-UP

A widely used sperm preparation technique in andrology labs around the world is the swim-up method. This simple initial step for in vitro sperm selection allows for the collection of the highest percentage of motile sperm in the top fraction, while low or non-motile sperm remain in the bottom fraction.

PROTOCOL:

1. Mix the semen sample well.
2. Place 1 ml of semen in a sterile 15-ml conical centrifuge tube, and gently layer 1.2 ml of medium over it. Alternatively, pipette the semen carefully under the culture medium.
3. Incline the tube at an angle of about 45°, to increase the surface area of the semen– culture medium interface and incubate for 1 hour at 37 °C.
4. Carefully put the tube back upright and take off the top 1 ml of medium. Highly motile sperm cells will be present in this.
5. Use 1.5 to 2.0 milliliters of medium to dilute this.
6. Centrifuge for five minutes at 300–500g, then discard the supernatant.
7. To evaluate sperm concentration, total motility, and progressive motility, resuspend the sperm pellet in 0.5 ml of media.
8. Direct use of the specimen for research or therapeutic purposes is possible.

(Ref : WHO Manual for the examination and processing of human semen 6th edition)

ADVANTAGES:
1. No physical damage to the sperms
2. Simple and straightforward
3. Cost-effective

DISADVANTAGES :
1. Can only be used for normozoospermic (with good count, motility, and morphology) samples
2. Compromised capacitation
3. Limited ability to remove the non-sperm cells like round cells, debris, and microbes etc

KEY INSIGHT :
Many laboratories are happy with the time honoured swim-up method for its simplicity and inexpensiveness.

2. MICROFLUIDICS (MF):
Microfluidics primarily utilizes **rheotaxis**, which is the movement of sperm in response to fluid flow. Sperm can navigate through the microfluidic channels by swimming against the flow, allowing for the selection of motile and morphologically healthy sperm. While capillary action may play a role in fluid movement within the chip, rheotaxis is the key principle that drives sperm selection in microfluidics. Studies show microfluidics yield high quality (DNA) sperms.

PROTOCOL:
1. The semen samples loaded in the inlet of the device
2. The medium is then loaded in the specified area of the device, and incubated for 15-30mins. Good quality sperms can be retrieved from the outlet.

(Protocol depends on different microfluidics devices per manufacturer's instructions)

AVDANTAGES:
1. Supposed to separate sperms with better DNA integrity.
2. No mechanical stress to the sperm
3. Claimed to be effective in cases of recurrent implantation failure, miscarriages, poor blastulation rate.
4. Good for all ART procedures

DISADVANTAGES:
1. Cannot be used for Severe OATS
2. Costly
3. Compromised capacitation.

KEY INSIGHTS:
Although microfluidics can be expensive, many centers are adopting its use as more affordable devices are becoming available.

3. DENSITY GRADIENT (DG):
Centrifugation is employed in the density gradient (DG) sperm preparation method to separate sperm from seminal plasma. This method is traditionally the most commonly used in ART labs. Gradients such as 45-90 or 40-80 can be utilized for effective separation.

PROTOCOL:
1. In a conical test tube, a layer of higher density (80/90%) gradient is put in the bottom.
2. Without combining the two gradients, add 1-2 mL of the upper phase (45%) on top.
3. Top with 1-2mL of liquid semen and centrifuge at300-500g for 15–20 minutes.

4. Without disturbing the pellet at the tube's bottom, remove the supernatant.
5. Transfer the pellet to a new conical tube with 2–5 ml of sperm wash medium.
6. Repeat the centrifugation for 10 minutes at 300-500g and discarding the supernatant.
7. Incubate the pellet for 15 to 20 minutes after re-suspending it in 0.1 to 0.5 ml of sperm preparation medium. *(Ref : Nanna, A. (2022).*

ADVANTAGES:
1. The most common and traditionally trusted method.
2. Cost-effective.
3. Produces a clear fraction of motile spermatozoa.
4. Ideal for seropositive samples.

DISADVANTAGES:
1. Mechanical stress due to centrifugation,
2. Low sperm recovery in OATS sample
3. Repeated centrifugation may increase the DFI

KEY INSIGHTS:
At the end of the discussion, many members favoured sticking to traditional methods, citing their cost-effectiveness. However, others pointed out that newer devices are delivering better results and can be used routinely as the prices have come down.

5. **HANDLING SURGICALLY RETRIEVED SAMPLES**

Surgically derived sperm may exhibit structural abnormalities, so, selecting the most normal-looking sperm help to increase the fertilization and pregnancy rates. Surgery can be performed on the same day as Oocyte Pickup (OPU) or the frozen spermatozoa from previous surgery can be used. Some experts suggest preparing these samples using density gradient centrifugation and washing. Employing collagenase and/or erythrocyte lysis buffer (ELB) to eliminate tissue and blood can help in finding the rare sperms.

Additionally, treatment of Theophylline can also be done to induce motility in completely non-motile sperms. Some members use Hypo-osmotic swelling (HOS) test as a classical adjuvant for selecting viable spermatozoa.

PROTOCOL (Collagenase)
1. Preheat the Collagenase to 37 °C. Collagenase is buffered by HEPES, so should not be kept in a CO2 incubator.
2. Carefully place the testicular tissue/tubules in the tube containing collagenase.
3. For 60 minutes, place the tube in the incubator (or, ideally, a heat cabinet for tissue digestion) with the tube entirely closed. Every 20 to 30 minutes, a little agitation will help the creation of a suspension of a single cell.
4. Carefully pipette the digested tissue up and down to suspend the free sperms and testicular tissue cells.
5. Wash the tissue cell suspension twice using 1-2 mL of HEPES-buffered sperm processing media by centrifuging it. Alternatively, a density gradient can be used to process the cell suspension.
6. Resuspend the pellet in a tiny volume of 30–80 µl of sperm processing media.

(Ref : GM501 collagenase - sperm processing - cell culture media. Planer. (n.d.). https://planer.com/products/gynemed/sperm-processing/gm501-collagenase.html)

KEY INSIGHT:
Collagenase and ELB aid in quickly finding the surgically retrieved sperms processing. Motility enhancers such as Theophylline and methods like HOS help in the selection of live sperms in case on total immotile sperms

6. **FROZEN SEMEN SAMPLE PREPARATION:**

If raw semen is frozen, they need to be processed by a suitable method as described above.(depending on the quality of sample).

Semen samples which are already processed before freezing, can be used directly for IUI without the need for washing. However, popular opinion suggests that getting rid of cryoprotectantby a simple wash or a combination of gradual dilution plus swim-up may be better to avoid any adverse effects.

Protocol for thawing:

1. Thaw the frozen vial at room temperature for 10mins & in dry incubator for 15mins (alternative in water bath at 37° for 15mins
2. Frozen straws, however should always be thawed in water bath at 37° for 6mins.
   Slowly add washing media, drop by drop with constant stirring of thawed sperm sample.
   (protocolmay vary between individual labs)

KEY INSIGHT:

Faster warming of frozen sample results in better survival. Gradual addition of wash medium reduces osmotic shock. Removal of cryoprotectant is essential to avoid allergic reactions if used for IUIs. It also results in better fertilization with cIVF/ICSI.

CONCLUSION:

Some organizations also utilize techniques like MACS or PICSI for sperm selection, and freezing by VD for cases of cryptozoospermia. Some embryologists preferred microfluidics (MF) over density gradient (DG) methods. Others noted that MF is nearly equivalent to PICSI for sperm selection. The debate continued over favouring traditional methods versus new technologies; however, there was no definitive answer as various factors were considered. Standard operating procedures (SOPs) were recommended to ensure consistent results. While the majority viewed MF as a promising technique, a few raised concerns about potential sperm incapacitation due to prolonged exposure, although there was insufficient data to support this.

For further reading

1. *GM501 collagenase - sperm processing - cell culture media*. Planer. (n.d.). https://planer.com/products/gynemed/sperm-processing/gm501-collagenase.html
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4. Agarwal, A., Cho, C.-L., Esteves, S. C., & Majzoub, A. (2017a). Implication of sperm processing during assisted reproduction on sperm DNA integrity. *Translational Andrology and Urology*, *6*(S4) https://doi.org/10.21037/tau.2017.04.20

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