"Different techniques of semen preparation for different semen parameters"

Dissertation submitted in partial fulfilment for the degree of

Master of Science in Biotechnology

Submitted By

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''कन्या बचाओं-बेटी बचाओं''
NEELKANTH - A Unit of NFWCHPL
Fertility & Women Care Hospital
"Realizing the dream of motherhood"

CERTIFICATE

This is to certify the dissertation entitled ***"Different techniques of semen preparation for different semen parameters"*** submitted by ***KIRAN KUSHWAHA*** in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology, KIIT School of Biotechnology, KIIT University, Bhubaneshwar bearing Roll No. ***1661013*** & Registration No. is a bonafide research work carried out by her under my guidance and supervision from ***December 9th 2017*** to ***May 1st 2018***.

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This is also certify that the above said work has not previously submitted for the award of any degree, diploma, fellowship in any Indian or Foreign University.

Date:

Place:

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(Senior Chief Embryologist)

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DECLARATION

I hereby declare that the dissertation entitled "***Different techniques of semen preparation for different semen parameters***" submitted by me, for the degree of Master of Science to KIIT University is a record of bonafide work carried by me under the supervision of, ***Mr. Vinod Saini***, ***Senior Chief Embryologist***, at ***Neelkanth Fertility & Women Care Hospital, Udaipur, Rajasthan, India.***

Date: 4.05.18
Place: *Bhubaneshwar*

KIRAN KUSHWAHA

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KIRAN KUSHWAHA

ABSTRACT

Reproductive technologies, particularly In- vitro fertilization (IVF), have provided great hope for infertile couples worldwide. Indeed, more than 7 million IVF babies have been born since the birth of the first child, Louise Brown, in 1978.

During processing, viable sperm cells are first separated from other constituents of the ejaculate as early as possible. If spermatozoa are not separated from seminal plasma within 30 minutes of ejaculation, the in vitro fertilization (IVF) capacity permanently diminishes.

The World Health Organization (WHO) recommends separating sperm cells from the seminal plasma within one hour after ejaculation to limit damage from leukocytes and other cells present in the semen.

This thesis will discuss the various sperm preparation and selection techniques used to process sperm for use with assisted reproductive techniques: swim-down, swim-up, migration-sedimentation, density gradient centrifugation. It will also explain the procedures used to prepare viscous semen samples as well as when to obtain and prepare semen samples using epididymal and testicular spermatozoa, assisted ejaculation, and retrograde ejaculation.

Above all these techniques used for different sperm parameters the Density Gradient with Swim-up is ideal for the semen washing/preparation for Normozoospermia sample and Pellet with swim up is ideal for severe oligozoospermia sample.

CONTENTS

LIST OF ABBREVIATIONS

ART- Assisted Reproductive Technology

IVF- In-vitro Fertilization

ICSI- Intra Cytoplasmic Sperm Injection

ROS- Reactive Oxygen Species

HTF- Human Tubal Fluid Media

HOSt- Hypo-osmotic swelling test

MESA- Microscopic Epididymal Sperm Aspiration

PESA- Percutaneous Epididymal Sperm Aspiration

TESA- Testicular Epididymal Sperm Aspiration

TESE- Testicular Epididymal Sperm Extraction

INTRODUCTION

Sperm preparation for in vitro fertilization and intra- cytoplasmic sperm injection is an important step for successful outcome of ART procedure. Various sperm preparation methods are available. The choice of a particular method depends upon the assessment of:

1. The absolute motile count
2. Volume of semen
3. Presence of agglutination, pus cell, debris and antibodies
4. Procedure planned

Sperm preparation is carried out in order to remove the seminal plasma, dead spermatozoa and other cells, as well as to select the best spermatozoa.

The choice of sperms preparation method is based on patient history and previous semen analysis as well as an examination of the present sample. Another consideration is whether the fertilization will be performed by in vitro fertilization (IVF) or intracytoplasmic injection(ICSI), as more spermatozoa are needed for IVF insemination.

Whichever method is chosen, the aim remains same, i.e., maximum yield of morphologically normal motile sperms without seminal plasma/ debris/ pus cells and with minimal DNA fragmentation. It has been demonstrated that presence of pus cells and dead sperms in the semen are source of reactive oxygen species which can be detrimental to sperm membrane integrity thus affecting sperm fusion events during process of fertilization.

There are two basic methods to choose from for ejaculated semen. Both methods can be practiced with various modifications depending on the quality of semen and requirement of procedure. Irrespective of method used, final sample should have at least 50,000 to 2,00,000 motile sperms for ivf insemination, with minimal dead sperms and debris. For, ICSI few motile, morphologically normal spermatozoa are sufficient, however the final preparation should be as clean as possible.

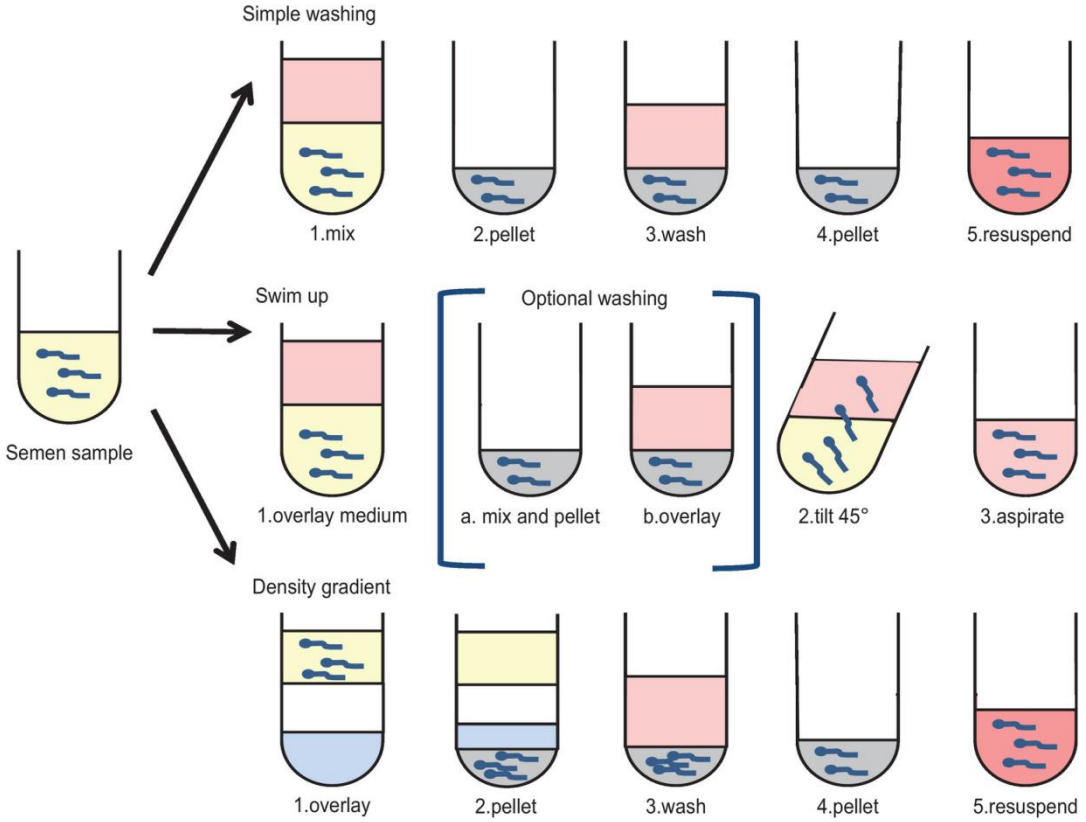


Fig. Different sperm washing technique

Sperm samples with moderate to high counts and good forward progression and motility can be prepared with pelleting and swim-up technique. Samples with poor forward progression, large amount of debris or anti sperm antibodies may be processed with discontinuous buoyant density gradient.

The initial sperm separation methods recommended one or two washing procedures with subsequent re-suspension of the spermatozoa or swim-up from the pellet. Latter, more sophisticated methods were developed to obtain sufficient amounts of motile, functionally competent spermatozoa for IVF. The aim was to develop an universal method for all types of semen sample and thus having improved sperm functions and reduced detrimental reactive oxygen species(ROS).

ESSENTIALS OF SEMEN PREPARATION

1. The sperm preparation should be performed in a clean aseptic work area. Nontoxic, non-powdered gloves and protective eyeglasses should be worn while handling semen samples.

2. All samples should be collected in appropriate sterile, nontoxic containers. It is recommended that the semen sample be collected not more than 1 hour before preparation. The semen sample should be protected from cold and heat.

3. All laboratory procedures, including a thorough identification protocol of the patient should be followed.

4. The first portion of the ejaculate contains most of the spermatozoa (high fraction) while the rest of the ejaculate mostly consists of secretion from the seminal vesicle (low fraction). It is therefore of the utmost importance that the first drops of the ejaculate are collected in the test tube provided for sperm collection.

5. Time from ejaculation to the beginning of sperm preparation should not exceed 60 minutes.

6. A normal semen sample liquefies within 30-60 minutes after ejaculation.

7. Incubating sperm in the seminal plasma for too long will reduce recovery of motile sperm and render the sperm unable to undergo the alterations that make them capable of fertilization.

8. It is not recommended to centrifuge raw semen as potential toxic round cells and dead spermatozoa will concentrate around the motile spermatozoa.

9. For viscous semen samples, sperm preparation medium can be mixed with the semen sample prior to the sperm preparation.

NOMENCLATURE- SEMEN VARIABLES(WHO)

Table 1:
Cut-off reference values for semen characterstics as published in consecutive WHO manuals

Semen characteristics	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010
Volume (mL)	ND	≥ 2	≥ 2	≥ 2	≥ 1.5
Sperm count (10^6/mL)	20-200	≥ 20	≥ 20	≥ 20	≥ 15
Total sperm count (10^6)	ND	≥ 40	≥ 40	≥ 40	≥ 39
Total motility (%)	≥ 60	≥ 50	≥ 50	≥ 50	≥ 40
Progressive motility	≥ 2	≥ 25%	≥ 25% (a)	≥ 25% (a)	≥ 32% (a+b)
Vitality (%)	ND	≥ 50	≥ 75	≥ 75	≥ 58
Morphology (%)	80.5	≥ 50	≥ 30	(14)*	≥ 4*
Leukocyte count (10^6/mL)	< 4.7	< 1.0	< 1.0	< 1.0	< 1.0

The common terms we use while preparing sample are enumerated below:
* Normozoospermia: 2ml semen
* Oligozoospermia : ≤ 15 mill/ml
* Severe oligozoospermia: 5 mill/ml
* Teratozoospermia: Less than 15% normal forms
* Aspermia: No ejaculate
*Azoospermia: No spermatozoa in the ejaculate
* Necrozoospermia: Only dead spermatozoa in the ejaculate
Oligo-astheno-teratozoospermia(OAT)

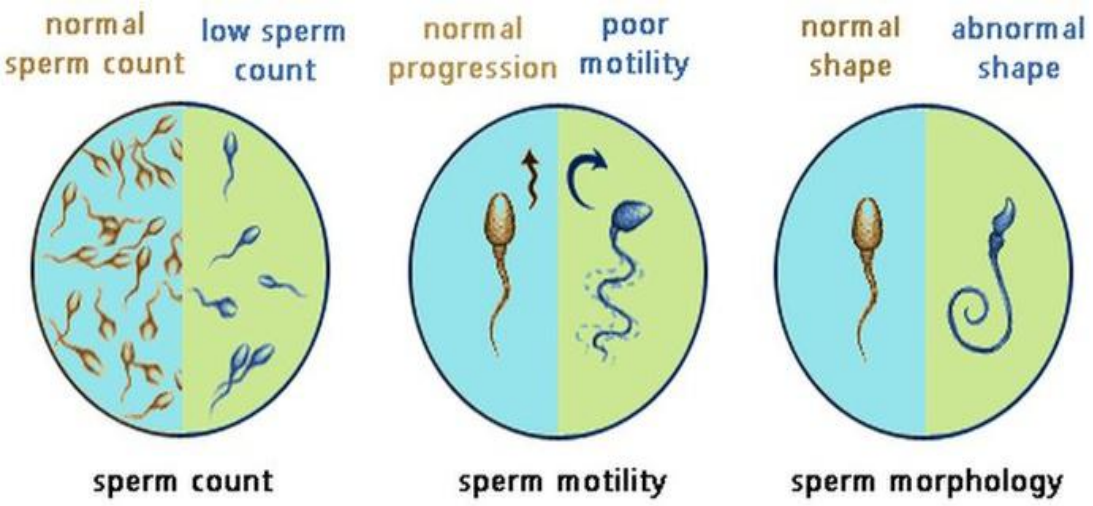


Fig: showing the 3 most important parameters for healthy sperm

GRADING OF MOTILITY

Rapid progressive motility(i.e.,≥ 25μm/s at 37℃)

Slow or sluggish progressive motility

Non-progressive motility

Techniques of semen preparation

Pelleting and Swim-up method

The method is good for normal semen parameters, with low volume or viscous samples. All preparations to be done after liquefaction before starting the procedure in following steps.

1. Mix equal volumes (1.5-2ml) of semen with prewarmed HEPES buffered HTF media in 5ml round bottom falcon tube. If volume is more, more tubes should be used.

2. Centrifuge at 1400 rpm for 10 minutes.

3. Carefully remove supernatant without disturbing the pellet.

4. Gently overlay 1 ml fresh HTF culture media over the pellet without disturbing the interface (gently shake the pellet if it is hard).

5. Incubate for 30- 45 minutes in CO2 incubator with cap loosen.

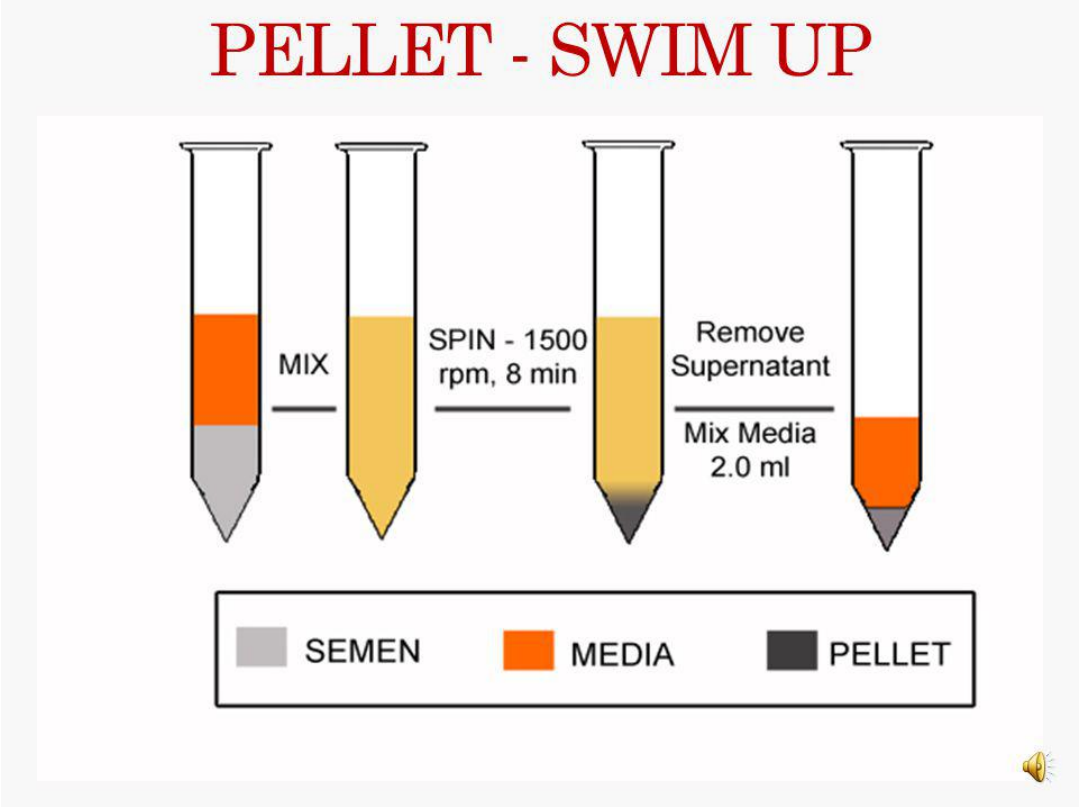
6. Carefully remove supernatant from all tubes in a clean 5ml round bottom falcon tube.

7. Centrifuge for 5 min. At 1400 rpm.

8. Remove supernatant leaving 0.3ml at the bottom.

9. Shake gently. Asses for count and motility.

10. Keep the tube in CO2 incubator till used for insemination with cap loosen (Dilute if high concentration)



DISADVANTAGE

This method has the disadvantage of exposing motile sperms to peroxidation damage during centrifugation with defective sperms and white cells as it is shown that defective and unselected sperms exhibit higher levels of reactive oxygen species production during centrifugation,

Swimup and wash

1. This method is commonly used for semen samples with normal sperm count/motility and selects the spermatozoa based on motility, ideally selecting only live sperms.

2. Aseptic technique should be used and semen should be processed within 1 hour of collection.

3. Allow approximately 20 minutes for liquefaction of semen. If the sample does not liquefy, you may need to pass it through a 23-gauge needle or a non toxic sterile narrow Pasteur pipette.

4. Make a microscopic assessment of the sperm sample to confirm the optimal method for processing the sperm. Use appropriate sterile microtips and pipettes.

5. Flushing medium/ sperm preparation media (MediCult), Gamete Buffer(Cook), sperm rinse (Vitrolife) is used for the initial swim-up. Warm and equilibrate the (sperm preparation/ sperm rinse medium) in a 6% CO2 incubator for a minimum of 4 hours prior to use. Other media have to be only warmed, as they are 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/ 3-(N-morpholino)propanesulfonic acid (MOPS) based.

6. Gently underlay (300µl) of fully liquefied semen under 0.5ml of equilibrated sperm preparation media(MediCult), fertilization media(Cook), sperm rinse(Vitrolife) in round bottom 5ml tubes.

7. Place tubes in a test tube rack so that the tubes are at 60° angle to the horizontal in the CO2 incubator. The test tubes are incubated at 37℃ for 30-60 minutes to allow the spermatozoa to swim up from the liquefied semen. Confirmation that sperm have successfully swam up into the overlaying culture medium is reflected by an increase in turbidity. If the culture medium appears clear, then more time may be needed to allow spermatozoa to swim out of the overlaid sample.

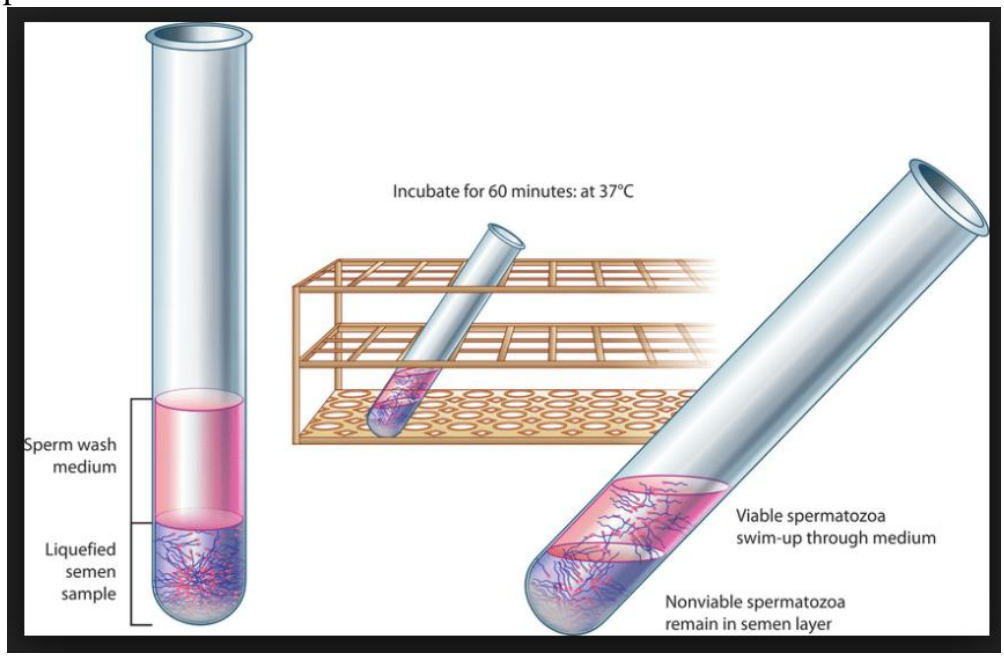
8. Remove the rack after 20-60 minutes and remove the medium above the semen sample (approximately 250µl of supernatant is removed).

9. Add 4.0ml of equilibrated universal medium (MediCult), G-IVF PLUS (Vitrolife) to the aspirated sample and centrifuge at 1,500 rpm for 10 minutes.

10. Remove the supernatant and resuspend the pellet in a small volume of equilibrated universal medium G-IVF PLUS (Vitrolife).

11. Count sperm and calculate the concentration.

12. Adjust the concentration as required and store the sample in a 6% CO2 incubator at 37℃ until required.



The principle of this conventional swim up and wash method is based on the active movement of spermatozoa from the sperm sample into an overlaying medium. Typically, the incubation time is 60 minutes. This technique has a recovery of a very high percentage (>90%) of motile sperm in the absence of contaminating dead or immotile spermatozoa, non-germ cells, and debris. The efficiency of the technique is based on the initial sperm motility in the ejaculate. The yield of motile spermatozoa is limited by the concept of pellet formation in the washing step. Many layers of cells in the pellet may not allow healthy motile spermatozoa in the lower levels of the pellet to reach the interface with the culture medium layer.

Density Gradient Centrifugation Method

This typical methodology for the density gradient centrifugation comprises of continuous or discontinuous gradients. In the continuous gradient technique, there is a gradual increase in density from the top of the gradient to its bottom, whereas the layers of a discontinuous gradient show clear boundaries between two medium, the ejaculate is placed on top of the density medium and then centrifuged for 15-30 minutes. During the centrifugation all cells reach the semen sediment layer. Highly active and motile spermatozoa move actively in the direction of the sedimentation gradient and can therefore penetrate the boundary quicker than less motile and immotile cells. Thus, we find that highly motile sperm cells are enriched in the soft pellet at the bottom. This method can be used to wash all samples of sperm regardless of quality.

Colloidal gradient is a solution stabilized with SpermGrad(Vitrolife) covalently bounded with hydrophilic silane coated colloidal silica particles in an isotonic balanced salt solution. By preparing different dilutions, solutions of different densities areobtained. Layering these solutions of different densities carefully in a centrifuge tube creates a density gradient. Cells and other particles with different buoyant densities will sediment until they reach a solution with higher density.

Centrifugation accelerates this sedimentation. We commonly use, a two- step gradient of 90% and 45% SpermGrad. Since mature sperm with tightly packed deoxyribonucleic acid(DNA) have a higher density than 90% SpermGrad, they sediment through this layer and are found at the bottom of the tube, whereas other cells, including immature and dead sperm, stop sedimenting at the 90% or 45% interface. SpermGrad is to be diluted in G-IVF PLUS for 90% and 45% gradients. G-IVF PLUS must be equilibrated at +37℃ and ^% CO2 before use. The final suspension of the pellet after the procedure is done in universal medium G-IVF PLUS(Vitrolife).

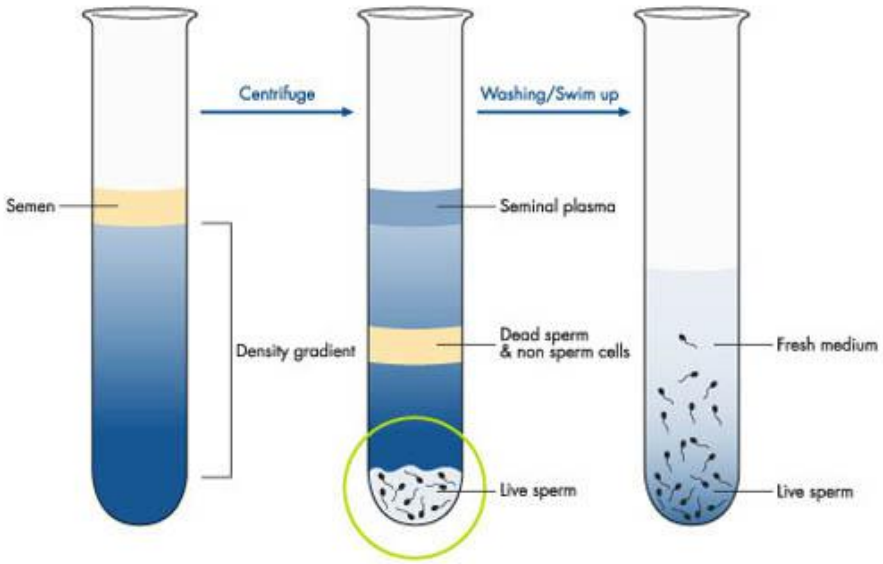


Fig. Density Gradient technique for semen sample

PROCEDURE OF SPERM PREPARATION BY DOUBLE DENSITY GRADIENT MEDIA (VITROLIFE)

1. Mix SpermGrad with supplement G-IVF PLUS in separate tubes to obtain 90% and 45% stock solutions. For 90% stock solution, mix 9.0ml SpermGrad with 1.0 ml supplemented G-IVF. For 45% stock solution, mix 4.5ml SpermGrad with 5.5ml supplemented G-IVF. Mix the solutions throughly and store in sterile nontoxic tubes or sterile tissue culture flasks. Label and refrigerate until use.

2. Always use a sterile nontoxic pipette to alliquot amounts needed for individual sperm preparations. Now do previous count of the sample. Stock solutions should be labeled with the date and kept for recommended time frames. Before use, allow the solutions to warm to ambient temperature and allow them to equiliberated in 6% CO2 to attain correct pH.

3. The density gradient should be layered in 2-4 sterile and rinsed conical nontoxic centrifuge tubes (depending on the volume of the semen sample) marked with patient identification(ID).

4. Pipette 1.5ml of 90% solution into the tube first and then slowly pipette 1.5ml of 45% solution on top of it. Finally, 1.0ml of the semen is gently layered on the top.

5. Make up 2-4 gradient tubes. Up to 2ml of semen can be layered on top of the gradient.

6. If the semen sample is of normal quality reduce the semen volume. Adding too much semen will result in poor separation.

7. The tubes are then centrifuged for 10-20 minutes at 300-600 g.

8. Remove the two top layers and take care not to leave any residue on the tube wall.

9. Transfer the sperm pellets with as little of the 90% solution as possible to a sterile conical rinsed tube with 5ml of equilibrated supplemented G-IVF PLUS and centrifuge for 10 minutes at 1500 rpm per 5 min.

10. Aspirate and discard the supernatants and repeat the wash. After the second wash, combine pellets and resuspended in 1ml of equilibrated supplemented G-IVF PLUS.

11. Dilute the washed sample with equilibrated supplemeted G-IVF PLUS to a final concentration of 75,000 -2,00,000 motile sperm/ml.

12. Alternatively, add equilibrated sperm suspension to equilibrated dishes with the oocytes already present. It is recommended to inseminate in a volume of 0.5-1.0 ml without oil overlay. If oil overlay is used, droplets of at least 100µl volumes are recommended.

Advantage of the density gradient method is the recovery of a higher percentae of morphologically normal spermatozoa than found in conventiona swim up or glass wool filtration. This method harvests spermatozoa from ejaculates with avery low sperm density. Sperms can be separated with good yields, round cells and dead sperms are eliminated to a large extent and ROS are significantly reduced in the semen sample.

The technique has also been shown to yield sperm populations with better DNA quality and chromatin packaging. Further, preliminary reports suggest that specimens known to be contaminated with sexually transmissible viruses can effectively be “cleaned up” using density gradient centrifugatio and the isolated spermatozoa can be used for therapy with exceptionally low risk for horizontal disease transmission.

One disadvantage of density gradient centrifugation is that the density gradient medium is a bit more expensive than either of the swim up techniques and there is a potential risk of endotoxins from the gradient constituents.

DOUBLE WASH AND SWIM UP

This method is commonly used on semen samples with normal sperm count/motility and selects the spermatozoa based on motility.

1. Two ml of semen sample after analysis is mixed with 4ml of sperm rinse(Vitrolife) or Gamete buffer(Cook) after the semen sample is liquified.

2. The sample and the media mixture are now transferred to the 15ml round conical tube (Falcon) and the sample is centrifuged for 10 minutes at 1,500 rpm. This step ensures that the sample is mixed with the media and the dead sperms and leukocytes and debris are separated.

3. Now the supernatant is discarded and the pellet resuspended in 2ml of universal medium(MediCult), fertilization medium(Cook) or sperm rinse medium(Vitrolife). Pellet is gently and throughly mixed with the above media and transferred to the 5ml round bottom tube(Falcon).

4. The sample is now spun for 5 minutes at 1,500 rpm in a centrifuge machine.

5. The supernatant is discarded and approximately 700µl of universal medium(MediCult), fertilization medium (Cook), G-IVF PLUS (Vitrolife) overlaid on the pellet.

6. The 5 ml (Falcon) tube is placed in rack and kept in the CO2 incubator at 37℃ for 20 minutes at an angle for the final swim up. Swim up from an intact sperm pellet requires that centrifugation speeds be such that the final pellet is loosely compacted. If we get a tight pellet the sperms may not swim up from it leading to lower yield. The consistency of the pellet may be verified by gently and slowly tilting the test tube and observing whether the pellet tilts or not.

7. Remember to keep the cap loose so that the sample can be easily equilibrated (exchange of CO2 can take place).

8. After 20 minutes the swim up is removed and placed in a fresh 5 ml tube for minimum 15 minutes for equilibration. The efficiency of the sperm swim up is based not only on the initial sperm motility in the ejaculate, but also on the size, level of compaction and exposed surface area of the final pellet.

9. Meanwhile perform the semen count and inseminating may be carried ot within 2 hours of removing the final swim up.

10. This technique remains my favourite and has given us persistently good resuts. It is easy to perform and reproduce.

Advantages of this method are that it is the simplest and the least expensive to perform. It guarantees recovery of a high percentage of motile sperm and absence of other cells and debris in the swim up. The swim up method also results in significant improvement in the rates of acrosome reaction, hypo-osmotic- swelling (HOS) and nuclear maturity.

CLINICAL TIP

It is important to understand that every semen has different characterstics and it is illogical to treat all specimen identically. All methods can be to tailored to fit the semen parameters.

SPECIAL SITUATIONS

Extremely oligospermic and asthenospermic samples cannot be processed by these routine methods and require specific approach depending on sample available.

Centrifugation and washing

In case of severely compromised samplewith occasion motile sperm.

1. Dilute the sample with media and centrifuge at 1200 rpm for 5 to 10 minutes.
2. Recover the pellet and resuspend in minimal volume(100-200µl).
3. Asses for the presence of sperm.
4. Put a small drop in centre (50µl) drop and overlay with mineral oil.
5. Pick up sperm by injection pipette for ICSI.

Swim out under oil

If lots of debris in final preparation than prepare a large droplet (100µl) of media, overlay with oil and place 20µl of final sample in the center of droplet and incubate for 30 to 60 minutes. Carefully aspirate top droplet and look for motile sperms for injection.

Obstructive Azoospermia: Epididymal and Testicular Sperm

Epididymal sperm can be obtained by MESA (Microscopic Epididymal Aspiration) or PESA (Precutaneous Epididymal Sperm Aspiration) by using 21'g' butterfly needle to aspirate fluid. If largenumber of sperm is obtained, can be processed by swim up or buoyant density gradient. If only few sperm are founded, the sample is washed and with fresh media and resuspended in minimal volume of media. A drop is put in injection dish. Under oil and sperms are picked up using injection pipette.

Testicular sperms can be obtained by TESE (Testicular sperm extraction- Open method) or by TESA(testicular sperm aspiration) using butterfly needle.

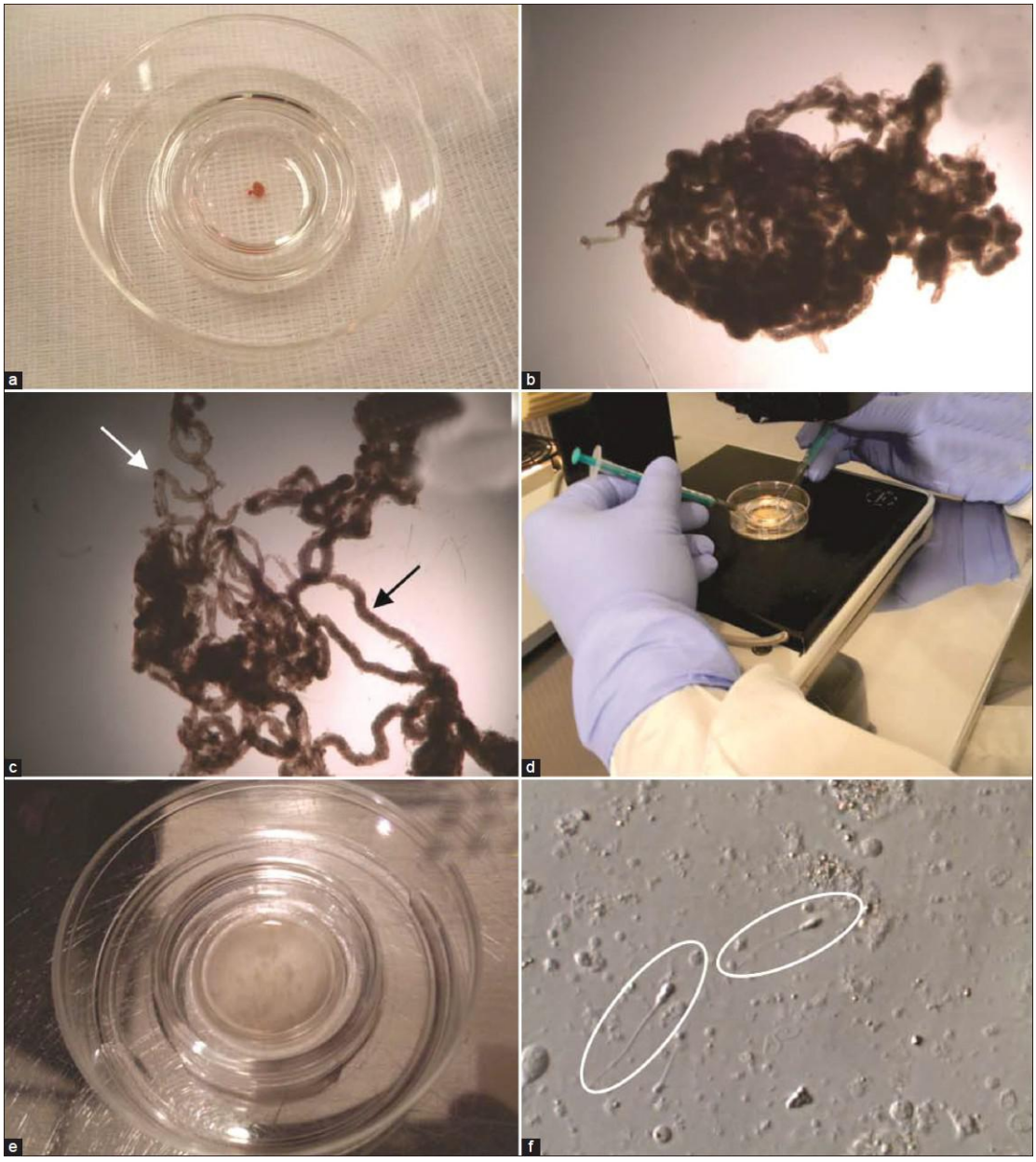


Fig: Step by step procedure of crushing the specimen

Specimen obtained is crushed between two slides or by fine scissors or by a fine needle. Further processing is similar as for epididymal sperm depending on number of sperms obtained. If no motile sperm are present, sperm are suspended in a fresh drop and observed under phase contrast- Micromanipulator for occasional twitching of tail and picked up for ICSI.

Alternatively, sperms are put in a hypo-osmotic solution drop and observed for curling of tail suggestive of viability. Such sperms are picked up and washed in fresh media drop before used for ICSI.

RETROGRADE EJACULATION

In cases of retrograde ejaculation, alkalization of urine is carried out by Alkalizers 48 to 72 hr before the procedure. Before ejaculation, bladder is catheterized and emptied.

Approximately 20 ml of media is instilled in the bladder. After ejaculation, bladder is emptied and sample centrifuged. Pellet is resuspended in fresh media and processed on buoyant gradient media for IVF or ICSI.

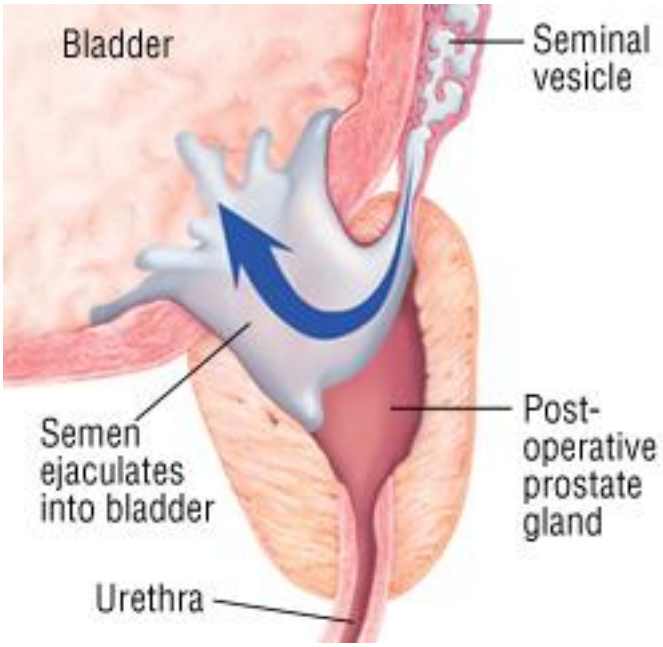


Fig. Arrow showing the pathway of semen from seminal vesicle towards bladder

HYPO- OSMOTIC SWELLING TEST (HOS)

Sperm motility is an important indicator of viability, especially when performing ICSI. When the testicular/ epididymal/ ejaculated sperms are immotile the assessment of viability becomes critical and unpredictable. HOS test is a simple vitality test based on the semipermeability of the intact and physiologically functional plasma membrane which causes spermatozoa to swell under hypo-osmotic conditions, when an influx of water results in an expansion of cell volume.

When setting up a dish for the ICSI procedure, a small (5µl) drop of HOS solution is placed near the polyvinylpyrrolidone (PVP) drop and two extra drops of culture medium are placed nearby. A small volume of sperm suspension is placed in one of the extra drop. When spermatozoa are located, they are aspirated up in the ICSI micropipette and placed in the HOS solution.

Immediately after the contact with the hypoosmotic medium, the tails of some spermatozoa will begin to coil or swell. Tail swelling or curling indicates that the immotile sperm is viable. HOS positive sperm is picked up in the ICSI micropipette and placed in the other extra drop of HEPES based medium in order to wash off excess hypoosmotic medium from both the micropipette and the spermatozoon. After rinsing the sperm in the fresh media droplet. It is placed in the PVP drop and ICSI can now be carried out.

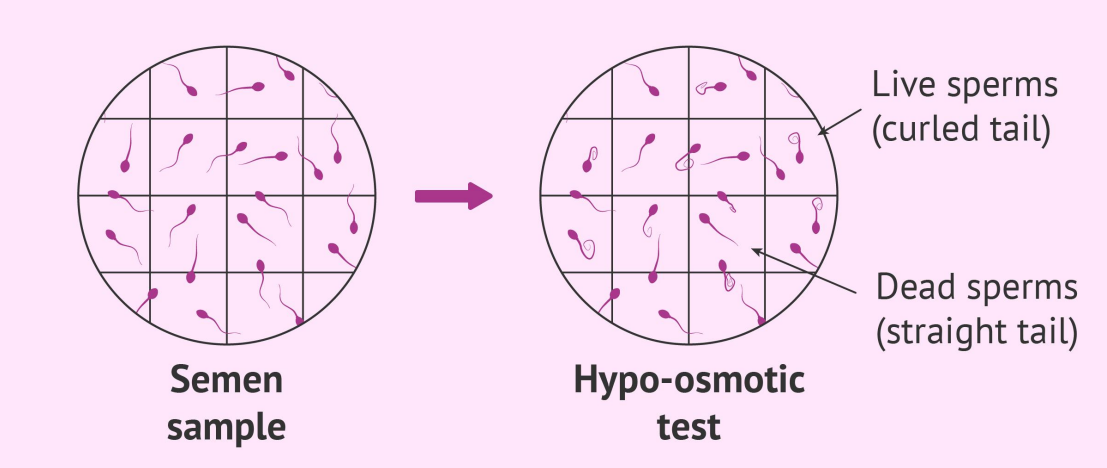


Fig. Semen sample after HOSt

THE IDEAl SPERM SEPARATION TECHNIQUE

The ideal sperm separation technique should:

I. Be fast, easy to perform and cost- effective.

II. Isolate as much motile spermatozoa as possible.

III. Not cause sperm membrane damage of the sperm cells.

IV. Eliminate dead spermatozoa and other cells, including leukocytes and bacteria.

V. Eliminate and not generate toxic substances like ROS.
Till date, we do not have any ideal method of sperm preparation and we have to select the available method from the various options available depending upon the semen sample.

SEMEN PREPARATION IN IUI

1. Prior to IUI, it is necessary to remove seminal plasma to avoid prostaglandin- induced uterine contractions.
2. Insemination with unprocessed semen is also associated with pelvic infection.
3. A variety of methods have been developed to separate the motile sperms from the ejaculate. The most common methods are washing and centrifugation which has been shown to cause some damage to the sperm.
   * Simple sperm wash
   * Swim up
   * Gradient
4. All preparations should done in a laminar flow for sterility.
5. The clean sperm suspension is used for IUI.

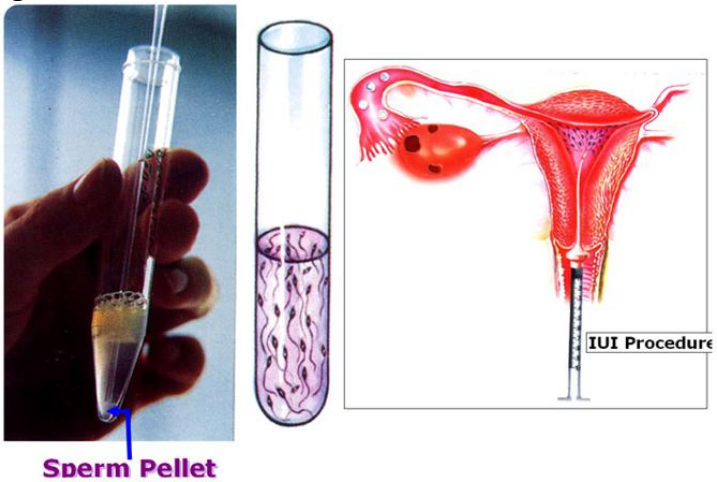


Fig. Highly motile sperm recovery for IUI

CASE STUDIES

A total of 150 patients sample were studied throughout their IVF cycles. Out of which 128 patients have normal sample (Normozoospermia) and rest have abnormalities in their sample. We wash these samples by using density gradient and swim up method. The results were noted as follows:

Normozoospermia/ Asthenozoospermia sample:

Total: 128

Method used:

Density Gradient recovery rate: 83/128 = 64.84%

Swim up recovery rate: 45/128 = 35.15%

By using both method together: 126/128 = 98.44%

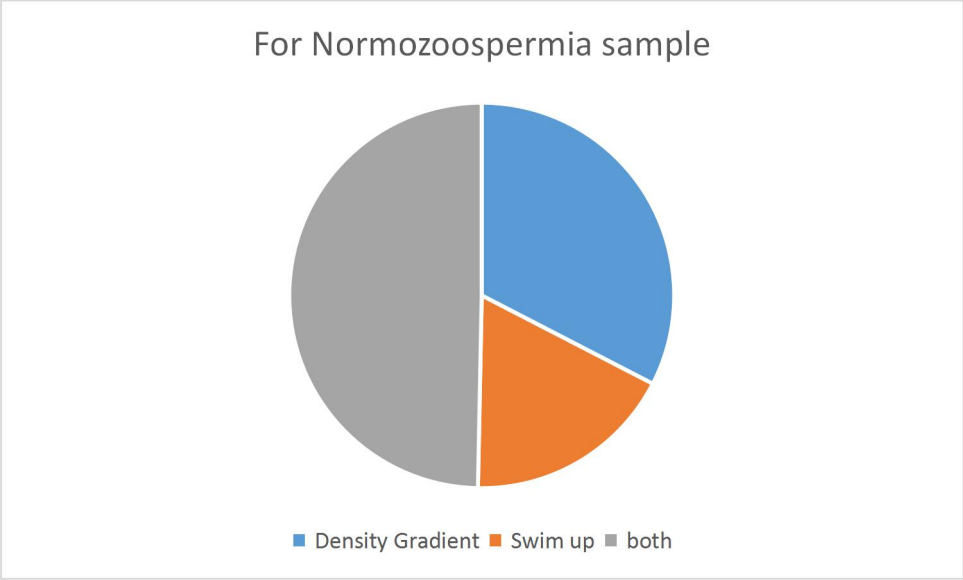


Fig. Pie chart showing the overall result

Severe Oligozoospermia sample:

Total: 18

Method used

Density Gradient recovery rate: 2/18 = 11.11%

Pellet and swim up recovery rate: 15/18 = 83.33%

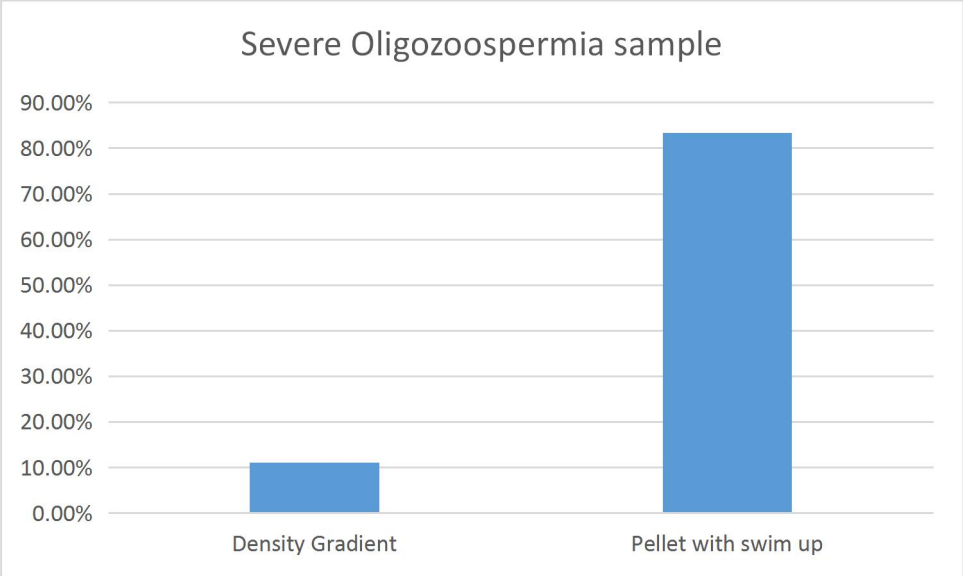


Fig. Bar Diagram showing the overall results

RESULT AND DISCUSSION

From the above case studies, it can be clearly stated that the outcomes, results and highest recovery rate of live or motile sperm can be achieved by sperm gradient with swim up for normal semen sample and pellet and swim up is best for severe abnormal samples.

But that doesn't mean that other method is useless or less effective if we don't have media for density gradient then just go for swim up method. It will give you at least some amount of semen sample. There is a line 'something is better than nothing' & this will fit in this case where you don't have any option for using this alternative step.

CONCLUSION

The choice and application of the appropriate sperm preparation technique can be a major contributor in influencing the quality of sperms used for IVF. Ejaculates from infertile / sub-fertile males commonly have the potential for producing ROS, which are known to compromise sperm function and damage DNA.

Therefore, it is imperative that the laboratory technician selects a technique that will directly separate functional and highly motile spermatozoa from all that remains. Density gradient centrifugation with swim up is the ideal and most effective method for semen preparation not all has ability to perform this test.

Thus, depending on the initial sperm parameters the direct swim up from semen can safely and effectively be used for semen preparation. We have to remember that final outcome of IVF depends upon the type of semen preparation, thus this critical step should never be ignored.

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