IFS CONVERSATIONS
Volume 15
Recent Advances in Sperm Selection Techniques for IVF

Recent Advances in Sperm Selection Techniques for IVF

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MESSAGE FROM THE PRESIDENT DESK

Dear Friends,

It is indeed a pleasure to address you all on this issue of IFS Conversations.

After the very successful virtual “Fertivision 2020”, I invite you all for “Fertivision 2021” which would be in a hybrid mode on 10th, 11th and 12th December with 12 pre conference workshops on 4th and 5th December 2021.

In this IFS conversation we have dealt with detailed analysis of the sperm factor in infertility and recurrent pregnancy loss. The editorial team and the authors have worked very hard towards it. Hope you all will find it very useful. The conversation also showcase various recent academic activities conducted by our extremely enthusiastic and committed members spread over 27 chapters across India and abroad.

Wishing you all a very Happy New Year!

Dr. Sudha Prasad
President- IFS

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MESSAGE FROM THE SECRETARY DESK

Dear Members and Friends

Indian fertility society has progressed over the past few years with almost 3500 members and 28 chapters. We are a very academically oriented society with monthly publications of newsletters and quarterly publication of IFS conversation. Even during the past 1.5 years when the whole world came to a standstill due to unprecedented corona pandemic, IFS was very active in continuing the knowledge updation through its various online academic activities.

This time the IFS conversation has focused on role of sperm DNA fragmentation in unexplained infertility and recurrent pregnancy loss. Also, there is a review on various latest sperm selection techniques. I congratulate the editorial board for their tireless efforts in bringing these publications throughout the corona pandemic.

I also take this opportunity to welcome you all for the 17th Annual National Conference, “Fertivision 2021”

I hope the knowledge provided in this issue of IFS conversation will be useful to you in your clinical practice.

Wish you all a very happy new year!

Dr. Neena Malhotra
Secretary - IFS
MESSAGE FROM THE EDITOR’S DESK

Dear Friends,

Greetings from team IFS

In this issue of IFS conversation, we present to you a very important, though often neglected aspect of infertility treatment, that is role of sperm testing beyond the routine semen analysis. Sperm is a contributor of 50% genetic material in an embryo. There have been recent studies focusing on role of sperm DNA integrity in successful implantation and beyond. In this issue, we present a case report with 2 times poor quality embryos followed by successful IVF with sperms selection via microfluidic technique. We have also reviewed the literature on role of sperm DNA fragmentation in cases of unexplained infertility and recurrent implantation failure. There is also an in-depth article focusing on latest sperm selection technology like Microfluidics, MACS, IMSI etc.

We are a very academically active society. This issue also highlights all the academic activities undertaken in last 3 months from our state chapters, SIG’s, vibrate webinars etc.

We sincerely thank all our authors for their wholehearted contribution towards this issue of IFS conversation. We would love to hear your comments and suggestions and also encourage all our readers to contribute in our forth coming issues of IFS conversations.

We look forward to meeting you all at Fertivision 2021.

Dr. Shweta Mittal Gupta
Editor, IFS

Dr. Rashmi Sharma
Joint Editor, IFS

INDIAN FERTILITY SOCIETY

IFS RECOMMENDATIONS FOR COVID 19 VACCINATION BEFORE ART

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Sperm DNA Fragmentation: A cause of repeated IVF-ICSI failure: A case report

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Joint Editor, Indian Fertility Society

Introduction

Infertility is a major issue among the couples. 1 out of 6 couples suffer from it. IVF has revolutionised the treatment of infertility but still there are many technical challenges to its success. Failure of IVF is a challenging situation. And if it occurs repeatedly it can have a major impact both on the patients and treating doctor. Overall success rate of IVF is between 30%-40% (1).

The pregnancy outcome after ART procedures has been unpredictable because several possible factors were involved in the process. Routine semen parameters, like semen concentration, motility and the percentage of normal sperm morphology are insufficient to predict pregnancy outcome after ART procedures(2).

Recent studies have shown that the integrity of genetic material in the sperm is essential for successful fertilisation and development of a healthy pregnancy. Sperm DNA fragmentation is a term used to denote abnormal genetic material within the sperm, which in turn may lead to male subfertility, implantation failure and miscarriages. Sperm DNA fragmentation (SDF) testing measures the quality of sperm DNA and sperm tail carrier, and it therefore is more significant than the parameters analysed in conventional semen analyses.(3)

DFI known as sperm DNA fragmentation index, was established to evaluate sperm chromatin integrity, and has gained increasing application for its diagnostic capabilities of male fertility potential and pregnancy outcome (4, 5)

While fully protaminated sperm DNA is highly stable and resistant to damage, deficiencies in protamination leave the DNA poorly compacted and more prone to damage (6)

Higher SDF is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in non-factor male infertility intracytoplasmic sperm injection cycles. Since defects in sperm may be hidden, the SDF test may bring additional information to the sperm quality evaluation of men with unknown infertility history(7).

Repeated implantation failure (RIF) is diagnosed when good-quality embryos repeatedly fail to implant after transfer in multiple IVF treatment cycles. There is no yet universally accepted definition for RIF, despite there is as yet no universally accepted definition for RIF, despite many publications on this topic (8). RIF may be due to defects with either endometrium or embryo. There have been lot of focus on female part of it but since embryo is 50% male contribution, there have been recent focus in evaluating the role of sperm DNA fragmentation in RIF

Case report

A young couple with married life of 5 years came to Origyn fertility and IVF centre, Pampura with primary infertility. The wife was 35 years old and husband 36 years old. The couple had regular cycles coming at an interval of 25-30 days and normal flow. Her baseline showed an endometrial poly of 12x8 mm size. AMH was 2.3 ng/ml, other investigations were normal. HSG showed bilateral patent tubes.

His semen analysis showed severe oligoasthenospermia with sperm count < 1 million, 12 % showing forward motility and 2% morphologically normal sperms. Repeat semen testing again showed sperm count< 1 million, 11% forward motility and 2% morphologically normal sperms.

Hysteroscopic polypectomy was planned for wife followed by IVF-ICSI.

In the first cycle, ovarian stimulation was achieved with an antagonist protocol. On cycle day 2, recombinant FSH and human menopausal gonadotropins administration was started. Their doses were adopted according to the number of ovarian follicles and their growth rates in vaginal ultrasonography. Gonadotropin-releasing hormone antagonist (GnRH antagonist) was initiated on 6th day and continued up to the day when at least three follicles reached a diameter of ≥18 mm. Dual trigger was given and after 35 hours, OPU was performed. 13 oocytes - 5 M1 AND 8 M2 oocytes were obtained. ICSI was done and 5 grade B embryos were seen on day 3, only 1 blastocyst could be transferred on day 5. Beta HCG 12 days later confirmed negative result. Patient came back after second IVP failure from a different centre. In this cycle also in spite of good number of M II oocytes, only 1 not so good blastocyst was available for transfer.

Both these cycles resulted in poor quality embryos.

Sperm DNA fragmentation test was done after 2 IVF failures with a high DFI score of 7.2% where normally it should be <30%. Husband underwent varicoce surgery after consultation with a urologist. He also was put on antioxidants for 3 months. After 4 months – DFI score again was high at 60% along with continuing poor sperm count and motility.

Patient underwent third cycle of IVF – ICSI with sperm selection with the help of microfluidic chamber . This time we could obtain good quality 7 embryos ( vitrified on day 4 – compaction stage in 3 straws)

2 grade A compaction stage embryos were transferred in the subsequent FET cycle with successful ongoing singleton pregnancy.

Discussion

Implantation is one of the most critical steps in reproduction. Recurrent implantation failure (RIF) is the absence of implantation after repeated embryo transfers. While this clinical phenomenon is commonly encountered and there is vast literature on the subject, there is no universally accepted definition.

Apart from the well-known causes for RIF still sometimes the cause remains not known. Studies at molecular level can explain many unexplained causes of RIF. Sperm DNA fragmentation is one on them.

Many studies have shown varying results between sperm DNA fragmentation and recurrent IVF failure with some studies reporting it is the cause and some saying it to be important cause of RIF (9)

Study by Coughlan C et al., do not support the hypothesis that sperm DNA fragmentation is an important cause of RIF or recurrent miscarriages, or that sperm DNA integrity testing has value in such patients(10).

Study by Hao J et al, indicate that assays detecting sperm DNA damage should be recommended to those suffering from recurrent failure to achieve pregnancy. Selection of sperm without DNA damage for use may improve the clinical outcome of ART(11).

Debate still goes on. For our patient very high sperm DNA fragmentation appears to be important cause of repeated failure to obtain good quality embryos and IVF failure. Selecting sperm with probably intact DNA through the use microfluidic chamber in this case seems to have helped the couple.

References:


Sperm DNA fragmentation: A hidden cause of unexplained infertility and recurrent pregnancy loss

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Introduction

Unexplained infertility and recurrent pregnancy loss are two areas in the field of ART which if present can bring much stress to both the treating doctor and the patient.

When in an infertile couple the results of a standard infertility evaluation, semen analysis, tubal tests and ovulatory studies are normal, a diagnosis of unexplained infertility is made. The likelihood that all such test results for an infertile couple are normal (i.e., that the couple has unexplained infertility) is approximately 15% to 30% (1).

Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies. The exact prevalence of RPL is difficult to estimate, but most studies report that RPL affects 1–2% of women [2].

Sperm DNA fragmentation denotes abnormal genetic material within the sperm, which in turn may lead to male subfertility, implantation failure and miscarriages. Since the male gametes lack many DNA repair mechanisms, sperm DNA is especially susceptible to damage by oxidative stress from neighbouring immature sperm, leukocytes, and other environmental exposures.

DNA damage, such as fragmentation and denaturation, can have an untoward effect on fertilization and embryo development and can cause infertility and even recurrent pregnancy losses [3]. Sperm DNA fragmentation (SDF) testing measures the quality of sperm as a DNA package carrier, and it therefore is more significant than the parameters analysed in conventional semen analyses [4]. Sperm DNA integrity testing has therefore been proposed to be a test with promising potential to complement the standard semen analysis [5].

This review of literature focuses on sperm DNA integrity testing in the evaluation of unexplained infertility and recurrent pregnancy loss.

What is sperm DNA fragmentation and why it happens?

To simplify, sperm DNA fragmentation occurs when there is a damage in the base or a physical break in one or both of the DNA strands of the chromosomes contained within the sperm. Besides single standard DNA breaks (SS-Ds) and double standard DNA breaks (DS-Ds), chromatin damage includes altered chromatin configuration and defective nuclear protein.

Sperm DNA integrity is essential for the birth of healthy offspring [6]. Increasing documentation shows that sperm DNA fragmentation (SDF), a sign of damaged chromatin, has an independent and remarkable role in male infertility and reproductive success [7].

The causes of sperm DNA damage are numerous, of complex nature and could be testicular or post-testicular [8]. These may include defects in

spematogenesis (e.g., genetic or developmental abnormalities) and testicular or post-testicular injury (e.g., gonadotoxins, hyperthermia, oxidants, and endocrine abnormalities). It has been suggested that protamine deficiency (with consequent aberrant chromatin remodelling), reactive oxygen species and abortive apoptosis may be responsible for sperm DNA damage [9].

A review article by Alaa hamada et al. on unexplained male infertility: diagnosis and management, concluded that further tests are certainly required beyond semen analysis for evaluating sub-fertile couples where cause remains unknown. The potential role of molecular developments in the field of andrology to bring robust and cost-effective clinically useful tests to look for sperm DNA fragmentation to fix the shortcomings of the routine semen analysis [11].

Study by Kim YG et al concluded that physicians and researchers working with ART must continue to make efforts to obtain healthy sperm with nuclear DNA integrity to minimize the adverse effects that may arise in offspring conceived from sperm with DNA damage [12].

A review of literature by Panduranga S et al says that though semen analysis is the cornerstone of evaluating male infertility, it is imperfect and insufficient to diagnose male infertility. As a result, about 10–40% of infertile men fall into the category of unexplained infertility, a term encompassing male infertility with an unknown underlying cause [13].

Thus we see that it may be advisable to offer sperm DNA fragmentation testing to couples with unexplained or idiopathic infertility. An abnormal test result may indicate that fragmented sperm chromatin might be the cause of infertility. In an age when a small quantity of sperm can lead to pregnancy through in vitro fertilisation or intracytoplasmic sperm injection, selecting healthy sperm is important. In couples with unexplained or idiopathic infertility, elevated sperm DNA fragmentation, a reproductive andrologist evaluation is warranted to assess underlying causes of DNA fragmentation.

Recurrent pregnancy loss

Recurrent pregnancy loss is a devastating experience for a couple and their caregivers. The aetiological basis of recurrent pregnancy loss (RPL) is heterogeneous.

Various causes such as uterine anatomical anomalies, genetic factors, and infectious and endocrine disorders have been reported for RPL. However, approximately 50% of the causes are unexplained, which may be due to male factors. Several studies have been done on semen parameters to determine the unknown causes and risk factors for miscarriages, however, studying condition semen parameters have not been sufficient.

Two very recent comprehensive reviews on sperm DNA fragmentation tests [14,15] have reopened the debate over their usefulness in improving pregnancy outcome.

In this regards, two aspects need to be seen. First, spermatozoa are not simply carriers of paternal chromosomes, but a role beyond fertilization. For instance, the spermatozoon transcribes genes critical for early embryonic development, inferring that integrity of sperm genome is essential for a successful gestation. Second, if sperm factors play a role in early embryonic development, are sperm DNA integrity tests useful as diagnostic and prognostic markers, especially in the context of recurrent pregnancy loss (RPL)? [16]

A systematic review and metaanalysis by McQueen DB et al concluded that there was a link between sperm DNA fragmentation and recurrent pregnancy loss. Further prospective studies were included in the review. Pooled data from 13 studies suggest that male partners of women with a history of recurrent pregnancy loss have a significantly higher rate of sperm DNA fragmentation compared to the partners of fertile control women [p<0.001 95% CI 1.49–18.86]. However, given the significant heterogeneity between studies, a lack of robust prospective pregnancy outcome data, they said that further large prospective studies are needed [17].

A study was carried out by Carlini T et al to investigate the male factor in Italian couples experiencing RPL following natural conception. The results suggested a correlation between increased SDF and impaired implantation in terms of both fertilization and pregnancies carried to term but they concluded that high SDF cannot be considered a predictive factor for the risk of RPL [18].

A meta-analysis was conducted of 12 prospective and 2 retrospective studies involving 5300 men with a history of RPL by Tan et al. In the study it was found that couples with a history of idiopathic RPL demonstrated higher levels of sperm DNA fragmentation compared to couples (average mean difference 11.98, P < 0.001). Results supported the diagnostic value of SDF over standard semen analysis, as well as a possible paternal derived genetic origin of unexplained RPL [19].

Not all the patients require sperm DNA fragmentation. It is mainly warranted in couples with

- Unexplained infertility
- Arrested embryo development
- Poor blastocyst development
- Multiple failed IVF/ICSI treatments
- Recurrent miscarriage
- Advanced chronological age
- Varicocele
- Poor semen parameters
- Exposure to harmful substances

What Does Testing for Sperm DNA Fragmentation involve?

There are many different tests for sperm DNA fragmentation, but the most commonly studied ones are:

1. The sperm chromatin structure assay (SCSA)
2. The deoxyribonuclease transfected-mediated dNTP nick end labelling assay (TUNEL)
3. The single-cell gel electrophoresis assay (COMET)
4. The sperm chromatin dispersion test (SCD)

These tests provide an estimate of the degree of DNA damage present in a semen sample.

Sperm DNA fragmentation testing warranted in unexplained infertility and recurrent pregnancy loss patients

It is found that in recurrent pregnancy loss and unexplained infertility patients, sperm DNA fragmentation examination could help to identify the cause of the infertility or pregnancy loss and guide the possibly therapeutic strategies.

Various studies have shown varying results regarding using sperm DNA integrity testing in infertility workup.

Unexplained infertility

When infertility couples or individuals have undergone all appropriate tests and no cause for their infertility is found, they are diagnosed with unexplained infertility. As already stated earlier, about 15–30% of infertile couples are diagnosed with unexplained infertility, also referred to as idiopathic infertility.

Unexplained infertility most likely involves issues with poor egg or sperm quality, or problems with the uterus or fallopian tubes that aren’t identifiable in routine infertility tests. Studies at molecular level have found out that DNA fragmentation can be the underlying hidden causes in some of these couples.

A study by Oleszcuk et al was done to investigate the prevalence of high DNA in male partners of unexplained infertility. In this study, the percentage of couples with diagnosis ‘unexplained infertility’ in which the male partner has DNA >20% or DNA >30% was calculated. In the group diagnosed with ‘unexplained infertility’ 17.7% of the men (95% CI 1.08–2.45) presented with DNA between 20 –30% and 6.4% (95% CI 3.40–13.4) had DNA ≥30%. A significant part of men diagnosed as unexplained infertility according to traditional diagnostic methods had remarkably high degrees of fragmented sperm DNA [10].

Various causes such as uterine anatomical anomalies, genetic factors, and infectious and endocrine disorders have been reported for RPL. However, approximately 50% of the causes are unexplained, which may be due to male factors. Several studies have been done on semen parameters to determine the unknown causes and risk factors for miscarriages, however, studying condition semen parameters have not been sufficient.

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Sperm DNA fragmentation: A hidden cause of unexplained infertility and recurrent pregnancy loss

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INTRODUCTION

Out of the millions of sperms that are ejaculated, only a few hundred of those make it to the ampulla, where they encounter the egg and fertilization takes place. Presumably, the selection of this subpopulation of sperms through the epididymis is done in a way that only the ones with highest fertilization capacity and all the required features to support embryonic development are selected to fertilize the egg.

One of the reasons for the low efficacy of ART has been credited to the lack of appropriate methodology and techniques that are simple, economical, ensure the enrichment of sample with best quality sperms which are devoid of leukocytes and bacteria, as well as toxic or bioactive substances like reactive oxygen species (ROS) are required. So, the techniques that meet all these requirements and are the most extended techniques for the preparation of sperms are Swim-Up (SU) and Density Gradient Centrifugation (DGC). Both these methodologies are used all around the world prior to IVF and ICSI, but the one of the major disadvantages of these procedures is that, the selection is only on the basis of the motility capacity of the sperms which does not necessarily mean that these sperms are of best quality. Therefore, the need of the hour are those sperm selection methods that are based on sperm characteristics which not only focus on the motility capacity of the sperm but also its morphology and fertilization ability.

Therefore, different sperm selection techniques are available, these includes PICSI, IMSI, MACS and MFSS. The application of these techniques depends upon sperm concentration, motility and the bottom of the dish (PICSI® Sperm Selection Device, MidAtlantic Diagnostic - Origo, Malmö, Denmark)

a) PICSI dishes are conventional plastic dishes, pre-prepared with 3 microdots of powdered HA which are then re-hydrated with ~5ul of fresh culture media

b) Then, a 2ul droplet of prepared sperm sample is then connected to the culture medium droplet along with the drops of Polyvinylpyrrolidone (PVP) are also placed elsewhere on the dish at this time for the manipulation of the sperms.

c) The CEP dish is then incubated under oil within 5 min and the spermatozoa are attached by their head to the surface of the HA-microdots and start spinning around their head. Finally, an ICSI injecting needle is used to pick up the best motile HA-bound sperms in order to inject them into the oocytes.

2. A viscous medium containing HA (Sperm Slow™, Medi-Cult – Origo)

This requires a specific method of droplet preparation in order to allow selection of sperms bound to HA. It is more versatile than PICSI as it can also be used on a glass bottom culture for higher magnification sperm evaluation.

Advantages

More varied filtered way of selecting the most competent sperms for ICSI process. In this, the choice of sperm is completely based on its ability to fertilize and perform the role

Disadvantages

There are no such drawbacks of this technique except for the fact that in case of TESA samples or immotile sperms wherein PICSI cannot be employed. It also cannot be employed in cases of patients with occasional sperm where sperm count is <1 million/ml

References


Intracytoplasmic Morphologically selected Sperm Injection (IMSI)

Principle
IMSI is a technique which involves the use of MSOME in conjunction with ICSI, the sperm selection criteria is based upon the various approaches used in studies to assess the sperm morphology through SEM (Scanning Electron Microscope) and TEM (Transmission Electron Microscope). The microscopic examination of sperms involves the assessment of six cellular organelles, wherein, in case of sperm head, three important characteristics taken into consideration are the shape, the presence of vacuoles and the base. So, MSOME does provide an accurate description of the sperm abnormalities, particularly the presence of head vacuoles (5). However, there has been no consensus on normal or abnormal MSOME criteria, despite being essential to transposing MSOME analysis into routine evaluation of male infertility (6).

History and Evidence
So, to overcome the limitations of the conventional magnification (sperm evaluation at ICSI is a maximum 400x), Bartooq et al. (1994, 2001, 2002) introduced a new approach involving real-time high magnification observation of untrained spermatozoa called Motile Sperm Organelle Morphology Examination (MSOME) and incorporation of this technique together with micromanipulation gave rise to a modified ICSI termed as Intracytoplasmic Morphologically selected Sperm Injection (IMSI). This method involves the use of an inverted light microscope equipped with high power Nomarski optic enhanced by digital imaging to achieve a magnification of up to 6300X.

But, according to a systemic review published in 2013 there is no evidence of effect of IMSI on live birth or miscarriage and the evidence that IMSI improves clinical pregnancy is also of very low quality (9).

Technique
The analysis of the sperm involves the use of interference phase contrast inverted microscope with the optics of Nomarski. The final image obtained on the screen is a result of a combination of the magnification of the objective, the camera adapter, ratio between the diagonal screen size (mm), diagonal of the camera chip size (mm), and internal magnification of the microscope. So, depending upon these specific characteristics of the IMSI system, the final magnification varies from 6000X to 66000X.

IMSI in practice
a) 1ul of prepared sperm sample droplet is placed inside the observation droplet of the prepared IMSI dish.
b) Sperms with morphological abnormalities are omitted and not aspirated while the motile sperms are transferred to the fresh PVP droplets and classified in accordance with classification published by Perdriz et al. (2012):
   • Type 0: spermatozoa without vacuole.
   • Type 1: spermatozoa with vacuoles occupying 0–5.9% of the nuclear surface.
   • Type 2: spermatozoa with vacuoles occupying 5.9–12.4% of the nuclear surface.
   • Type 3: spermatozoa with vacuoles occupying over 12.4% of the nuclear surface.

Finally, individual sperm cell is then placed in appropriate droplet based on the type in the IMSI dish.

Advantages
IMSI helps select live sperms in real time, therefore helps separate sperms
It allows for an accurate visualisation of sperms and is around 5 times more powerful than conventional microscopes used in IVF or ICSI

Disadvantages
It is very time consuming
Long exposure of sperm to the heated stage of microscope increases vacuolization in the sperm head and also affects the sperm cytoplasm

Magnetic Activated Cell Sorting (MACS)

History and Evidence
Evidence from various studies suggest that normal spermatozoa used for IMSI can have negative impact on ART outcomes. The methodologies like swim-up and Density Gradient Centrifugation do not take into account certain important molecular features such as apoptosis and/or sperm DNA fragmentation. Therefore, development of new technologies was required in order to allow better gamete selection. So, following this rationale, MACS was applied as a sperm preparation method in order to remove apoptotic cells using Annexin V Overall, it is a method of separating cells of interest from a mixed cell population.

Principle
MACS method is based on using of paramagnetic Annexing V-conjugated microbeads. It involves the conjugation of magnetic micro beads with specific antibodies or proteins on the target cell's membrane. It has been proposed as a safe method to select non-apoptotic and viable sperm (12). Annexin V has a strong affinity for phosphatidyl serine but cannot pass through the intact sperm membrane. Colloidal superparamagnetic beads (~50 nm in diameter) are conjugated to highly specific antibodies to annexin V and used to separate dead and apoptotic spermatozoa by MACS. Annexin V binding to spermatozoa indicates compromised sperm membrane integrity.

Technique
1) A 100 µl sperm sample is mixed with 100 µl of MACS micro beads and incubated at room temperature for 15 minutes.
2) The mixture is loaded on top of the separation column which is placed in the magnetic field (0.5 Tesla) between the poles of the magnet and 1.5 T within the iron globes of the column.1 Tesla = 10,000 gauss.
3) The column is rinsed with buffer. All the unlabelled (annexin V-negative) non-apoptotic spermatozoa pass through the column.
4) The annexin V-positive (apoptotic) fraction is retained in the column.
5) The column is removed from the magnetic field, and annexing V-positive fraction is eluted using the annexin V-binding buffer.

Disadvantages
Viable spermatozoa ought to be separated from all substances in the ejaculate such as apoptotic spermatozoa, leukocytes, and seminal plasma. MACS, which removes apoptotic spermatozoa, needs to be used in conjunction with other techniques such as density gradient centrifugation to remove the other substances.

Microfluidics

History and Evidence
The silicon microfluidics device was used in mid 1990s for selection of motile sperm (Kricka et al., 1993). Human semen was firstly processed in year 2003 and motile sperm isolation was done with impending therapeutic efficacy (Schuster et al., 2003). Ch. et al. 2003 designed special parallel microchannel maintained by gravity driven pumping mechanism in microfluidics device and Schulte et al., 2007 reported that microfluidic sperm processing could significantly increase the percentage of sperm with DNA damage and provide high motility sperm. Then, a microfluidic device was developed by Nosrat et al., 2014 to isolate progressively motile sperm in 500 parallel microchannel and also sperm with high DNA integrity and motility (15).[16]

Principle
Microfluidics helps sort sperms in a faster and a gentler way that closely mimics the natural sperm selection and avoids all the detrimental elements of current sperm sorting techniques. Microfluidics sperm sorter (MFSS) is a technique used to separate motile and morphologically good sperm with normal DNA integrity. It is a fluid dynamics-based model with sub microliter channels. The raw semen sample is used in this technique without any centrifugation, in order to avoid the generation of ROS. The geometry and flow involved in microfluidic platform closely mimics the natural in vivo locomotion of sperm at sub-microlitre level in micro confined environment of female reproductive tract.

Fig 3: Human spermatozoa with head vacuoles observed using MSOME, at >x6600 magnification (6)

Preparation of IMSI dish
The dish used for IMSI is usually a glass bottom dish in which three types of droplets are made.
   a) Observation droplets: These droplets of sperm culture medium might contain PVP ranging from 0% to 10% depending upon the intensity of motility of the sperms.
   b) Clean droplets of clean sperm culture medium: After the sperm cells have been evaluated, they are transferred to these droplets.
   c) Clean droplets of PVP 10%; These are 1ul droplets created parallel to the droplets containing spermatozoa. It is recommended to create bridges between these two droplets as it eases the detection of spermatozoa as it helps capture the head of mature sperms.

Fig 5: Magnetic Activated Cell Sorting (MACS)

Advantages
MACS acts at the molecular level as opposed to routine sperm preparation techniques that rely on sperm density and motility.

Disadvantages
Viable spermatozoa ought to be separated from all substances in the ejaculate such as apoptotic spermatozoa, leukocytes, and seminal plasma. MACS, which removes apoptotic spermatozoa, needs to be used in conjunction with other techniques such as density gradient centrifugation to remove the other substances.
4) Remove media from all the four chambers. Further, load 20 μl media in the chamber C and D, while 100 μl media in chamber B. Then, gently load 65 μl of suspended semen sample in chamber A.
5) The best motile spermatozoa move and get collected in chamber C within 30 to 35 minutes (25-30 μl).
6) While immotile spermatozoa, dead spermatozoa and debris accumulate in chamber D.
7) Sperm can be collected from chamber C and used accordingly for IVF/ICSI.

![Image of microfluidic device](image1)

**Microfluidic sperm sorting approaches can generally be sorted into three categories:**

**Type 1: Microfluidic devices that isolate only motile sperm**
This device employs the technologies that improve the swim up method by translating the process of motility screening to a microfluidic system and is one of the largest microfluidics devices.

![Image of microfluidic device](image2)

**Type 2: Microfluidic devices that isolate sperm cells without relying on sperm motility**
The selection mechanism of these microfluidics devices relies upon sperm shape, size, and/or other physical biomarkers instead of sperm motility. The primary focus of these systems is not on capturing an improved sperm sub-population, rather these systems focus more on the potential to retain the full fertilization capability of a sub-fertile semen sample by indiscriminately capturing sperm cells.

![Image of microfluidic device](image3)

**Type 3: Microfluidic devices for the observation and selection of individual sperm.**
These microfluidic devices take advantage of the ability of microfluidics to capture and non-invasively investigate the characteristics of a single sperm cell while maintaining sperm viability. An emerging area of research which focuses on isolation of a single sperm cell uses microfluidics sperm sorting system combined with Raman spectroscopy, a type of vibrational spectroscopy that relies on inelastic scattering of monochromatic light by the molecular structure of a system to determine the constituents of the system.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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</thead>
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<tr>
<td>Spermatozoa are separated without centrifugation that aids in reducing centrifugation induced ROS generation.</td>
<td>Can only work in progressive motility spermatozoa samples.</td>
</tr>
<tr>
<td>Spermatozoa separated have better DNA integrity.</td>
<td></td>
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**CONCLUSION**

The efficiency of Assisted Reproductive Technology (ART) still has a scope of improvement. Sperm selection is one of the most important factors on which the success of IVF and ICSI lies, especially in the cases where the cause of infertility is the male factor. However, the methodologies widely and commonly used have not proven to be useful and are effective in only certain cases of infertility.

But the various novel methodologies discussed above which are based on physiological selection operating in vivo and on microfluidic environment have given promising results.

But further studies are still required to get a better understanding of their advantages and limitations, in order to improve the ART outcome.

**Fig 6:** (A) Schematic of the mechanism of microfluidic sperm sorting devices; (B) Chip device made of cyclo-olefin polymer (Menicon Co. Ltd.); (C) Microscope images during sperm sorting; Yellow and orange circles show human sperm swimming across the interface of the two laminar flows (18)

**Fig 7:** Type 1- Microfluidic systems designed for separation of sperm based on sperm motility. (A) Motile sperm can be selected from immotile sperm due to their ability to swim across channel width (19); (B) motile sperm are selected and sorted by swimming speed using the imposed velocity gradient (20); (C) a series of parallel, long narrow channels are used to select motile, viable sperm (15)

**Fig 8:** Type 2- This figure depicts a microfluidic system designed for rapid separation of sperm from epithelial cells with application in forensics related to sexual assaults. (A) A picture of the actual device; (B) the cell mixture is aligned against the top wall in the pinched segment, and then the position difference of different sized cells is amplified in the expansion region; (C) sperm recovery rate is improved in the parallel capillary tubes
INDIAN FERTILITY SOCIETY STATEMENT
(14 April, 2020)

COVID-19 & FERTILITY
RECOMMENDATIONS FOR CLINICS & PATIENTS

For Details Visit
www.indianfertilitysociety.org
Rajasthan Chapter
Date: 19 June, 2021

Topics:
1. What is the effect of duration of abstinence before semen testing done?
2. Two labs show different semen analysis reports of the same person. How to identify correct report? What is a normal semen analysis report?
3. Ultrasound report shows the diagnosis of varicocele with subnormal semen parameters. What should be advised?
4. How to proceed if azoospermia is reported in the first semen analysis report and when and where to refer such cases?
5. Can we treat medically obese men with low semen analysis with sexual dysfunction?
6. What is the role of available antioxidants in treating low semen parameters in infertile men?
Questions faced by gynaecologist while treating infertile couples with semen abnormalities.

1. What is the effect of duration of abstinence before semen testing done?

2. Two labs show different semen analysis reports of the same person. How to identify correct report? What is a normal semen analysis report?

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Haryana Chapter
Date: 27 August, 2021

Punjab Chapter
Date: 8 September, 2021

Chhattisgarh Chapter
Date: 12 September, 2021
IFS ACTIVITIES 2021

SIG ACTIVITIES

IFS SIG- Applied Genetics
Date: 18 June, 2021

IFS SIG- Ultrasound
Date: 22 July, 2021

IFS SIG- Applied Genetics
Date: 6 August, 2021

IFS SIG- Applied Genetics
Date: 6 September, 2021
IFS ACTIVITIES 2021

**IFS SIG- Andrology**
Date: 19 September, 2021

**IFS SIG- Fertility Preservation**
Date: 26 September, 2021

**Indian Fertility Society**
IFS SIG

**FERTILITY PRESERVATION: EMERGING TRENDS**
Sunday, 26th September, 2021 | 10:15 AM - 12:30 PM

**Invited Faculty**

**Programme**

**Time** | **Topic** | **Speaker**
--- | --- | ---
10:00 - 10:05 AM | Welcome Address | Dr. Sudha Prasad
10:05 - 10:10 AM | Video Launching | Dr. Neena Malhotra
10:10 - 10:20 AM | Keynote Address | Dr. Pradeep Tewari
10:20 - 10:30 AM | Overview of Spermatozoa and its Fertility | Dr. Purna Rana Jana
10:30 - 10:45 AM | Fertility Preservation in Children, Adolescents and Young Adults (CAVA) Cancer Patients | Dr. PM Gopikrishan
10:45 - 11:00 AM | Full-Spectrum and Fertility Preservation | Dr. Reza Rezaii

**Session 1**
Chairperson: Dr. Neera Thandur, Dr. Santanu Chaudhuri, Dr. Ruhani Sharma

**Panelists**
Dr. Umesh Talwar, Dr. Sanzai Chandra, Dr. Ajay Agrawal, Dr. Beena Chatterjee, Dr. Pranay Chaturvedi, Dr. Sreejata Nandi

**Moderators**
Dr. Pradeep Tewari, Dr. Purna Rana Jana
IFS ACTIVITIES 2021
WEBINAR

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WEBINAR
**IFS ACTIVITIES 2021**

**WEBINAR**

**IFSS WEBINAR MEET 2021**

**Session 1**

**Topic:** Ovarian Reserve Management

**Speaker:**
- Dr. Shekha Prasad
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

**Chairpersons:**
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

**Session 2**

**Topic:** Optimising IUI

**Speaker:**
- Dr. Shekha Prasad
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

**Chairpersons:**
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

**Session 3**

**Panel Topic:** Difficult Shootouts in Infertility

**Panel Moderators:**
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

**Panelists:**
- Dr. Neena Malhotra
- Dr. Sarabjeet Singh
- Dr. Sukriti Bansal
- Dr. Jasleen Randhawa

Date: 18th August 2021 (Thursday)
Time: 4.30 PM to 8.30 PM

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**INDIAN FERTILITY SOCIETY WEBINAR**

**ART IN VIRAL INFECTIONS**

4th September, 2020 | 4:00 - 6:00 PM

**Welcome:** Dr. Sudha Prasad

**Panel Discussion:** Case Based Scenarios
- **Moderator:** Dr. Sudha Prasad & Dr. Neena Malhotra
- **Experts:**
  - Dr. KP Nayak
  - Dr. Kishore Shekhawat
  - Dr. Nidhi Goel
  - Dr. Anil Kamat

**Panelists:**
- Dr. Rakesh Verma
- Dr. Arpit Mehta
- Dr. Pratik Agarwal
- Dr. Rupal Shah

**Other Viral Infections**
- **Experts:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh
- **Panelists:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh

**Welcome Speech & Toast:**
- Dr. Sudha Prasad

**Join Link:**
- [Webinar Link](https://zoom.us/j/9352285000?pwd=OFREUGZ2S3ljTezAO9w3Vz0VYVYrND09)

---

**SVI O&amp;G Society in association with Indian Fertility Society, Navsari and Bhachar O&amp;G Society**

**Webinar on “Male Infertility”**

14th September 2021, Friday | 3:00 PM to 5:00 PM

**Welcome Speech:**
- Dr. Sudha Prasad

**Panel 1:**
- **Topics:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh
- **Panelists:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh

**Panel 2:**
- **Topics:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh
- **Panelists:**
  - Dr. Neena Malhotra
  - Dr. Sarabjeet Singh

**Welcome Speech:**
- Dr. Neena Malhotra

**Join Link:**
- [Webinar Link](https://zoom.us/j/9352285000?pwd=OFREUGZ2S3ljTezAO9w3Vz0VYVYrND09)
IFS ACTIVITIES 2021

WEBINAR

**IFS UTTARAKHAND CHAPTER**
Affiliated to Indian Fertility Society (IFS)

You are Cordially invited for Webinar

**Topic:** Role of Immunoglobulin in IUI

**Topic:** Role of testosterone gel in prediction and management of poor responders.

**Date:** 24th September 2021

**Time:** 3.00 to 5.00 PM

**Name of Activity:** IFS SIG Activity

**Date:** 13 September, 2020

**Name of SIG:** Endoscopy

**SIG Convenor:** Dr. Renu Mishra

**SIG Co-convener:** Dr. Damodar Rao

**SECRETARIAT**
IFS U.K. CHAPTER
Prasad Hospital, Adarsh Near Railway Station, Rishikesh

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**IFS U.P., RAJASTHAN & HARYANA CHAPTER**

**Presents**
Webinar Series: Master Class on - Living with PCOS

**Module 3:** Metformin vs. Inositol in PCOS

**Guest of Honour:**
- Dr. Sudha Prasad (President, IFS U.P.)
- Dr. Sonia Mallik
- Dr. Neena Malhotra (Secretary, IFS)
- Dr. Anju Mathur

**Speakers:**
- Dr. Babu Mukherjee
- Dr. Sunita Chandra (Secretary, IFS U.P.)
- Dr. Sonu Balhara (Secretary, IFS Haryana)
- Dr. Arjun Mathur (Secretary, IFS Rajasthan)
- Dr. Mukesh Gupta
- Dr. Anju Ran

**Experts:**
- Dr. Sweta Gupta
- Dr. Namita Kotia
- Dr. Neeti Tiwari

**Quiz Master:** Dr. Mangusha

**Judge:** Dr. Amrita Pandey

**Master of Ceremony:** Dr. Tripti Bansal

---

**INAUGURAL CEREMONY OF IFS HARYANA 2021 TEAM & FERTILITY CME**

**Date:** 24th June, 2021
**Time:** 4:30 pm – 6:30 pm

**Guest of Honour:**
- Dr. Sudha Prasad
- Dr. K D Nayar

**Speakers:**
- Dr. Priya Varshney
- Dr. Neenu Thakral
- Dr. Sonu Balhara
- Dr. Sudha Prasad
- Dr. Nalini Mahajan

**Experts:**
- Dr. Arjun Ran

**Judges:**
- Dr. Amrita Pandey

**Master of Ceremony:** Dr. Tripti Bansal

**Panel Discussion - ART Pregnancies & Role of ART Specialist in Prevention of ANC Complications**

**Moderator:** Dr. Neena Malhotra
- Dr. Aparna Sharma
- Dr. Sonia Malhotra (Expert)
- Dr. Sunita Chandra
- Dr. Anju Mathur, Dr. Ia Gupta
- Dr. Ritu Jain
- Dr. Vandana Chadha

**Question and Answer:**
- Dr. Shalu Gupta
- Dr. Seema Mittal

**Vote of Thanks:**
- Dr. Neena Malhotra

**Webinar Registration/Viewer link**
www.streamtech.in/IFS-Haryana-Chapter
IFS ACTIVITIES 2021

**GUT DYSBIOSIS & IMMUNITY: ITS ROLE DURING COVID-19**

**Name of Activity:** IFS SIG Activity
**Date:** 3 & 4 September, 2020
**Name of SIG:** Research Methodology

**SIG Convenor:** Dr. Mohan Kamat
**SIG Co-convener:** Dr. Ruma Satwik

**Panelists:**
- Dr. Saurabh Mulk
- Dr. Lalit Bharadwaj
- Dr. Kaysh Patel

**Supported by Enterogermina**

**WEBINAR**

**FERTILITY KONNECT**

For upcoming Gynecologists & Post Graduates on basics of Infertility & Reproductive Endocrinology

**Topic:** Evaluation of Female Partner - when, what & how much?

**Date:** 2nd & 4th Wednesday of the Month
**Time:** 14th & 28th July 2021, 7 to 8 pm

**Registration Link:** https://webinar365.in/SUN-SPECTRA-IFS-Webinar/

**Prior Registration is mandatory**

**MISCELLANEOUS**

**INDIAN FERTILITY SOCIETY CELEBRATES WORLD EMBRYOLOGIST DAY**

**INTERNATIONAL SYMPOSIUM**

**Date:** 25 July, 2021 | 8:00 PM - 9:30 PM

**INTERNATIONAL SYMPOSIUM**

**Date:** 25 July, 2021 | 8:00 PM - 9:30 PM

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**Date:** 25 July, 2021 | 8:00 PM - 9:30 PM

**INTERNATIONAL SYMPOSIUM**

**Date:** 25 July, 2021 | 8:00 PM - 9:30 PM
IFS ACTIVITIES 2021
MISCELLANEOUS

**IFS ACTIVITIES 2021**

**FERTILITY KONNECT**
For upcoming Gynaecologists & Post Graduates on Basics of Infertility & Reproductive Endocrinology

**Topic:** Beyond Basic investigations of male partners—For whom and how?

**Block:** 2nd & 4th Wednesday of the Month

**Date:** 11th August 2021

**Time:** 7 to 8 pm

**Registration Link:**
https://webinar365.in/SUN-SPECTRA-IFS-Webinar/
For more information: www.indianfertilitiesociety.org
Contact Number: +91 9899300983

**Name of Activity:** IFS SIG Activity

**Date:** 3 & 4 September, 2020

**Name of SIG:** Research Methodology

**SIG Convenor:** Dr. Mohan Kamat

**SIG Co-convener:** Dr. Ruma Satwik

**Name of Activity:** IFS SIG Activity

**Date:** 13 September, 2020

**Name of SIG:** Endoscopy

**SIG Convenor:** Dr. Renu Mishra

**SIG Co-convener:** Dr. Damodar Rao

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**Indian Fertility Society**

**Online Tutorials—Learn Laparoscopy**

**Who Should Attend:**
- Gynaecologists
- Infertility specialists

**Course Fees**
INR 2000

**Registration fees to be paid to: Indian Fertility Society**

**For Online Transfer**
- Account Name: Indian Fertility Society
- Account number: 918010102249295
- SWIFT Code: AXISINBBAA9
- Bank: Axis Bank
- Branch: Statesman House, Barakhamba Road, Connaught Place, New Delhi-110001

**Course Dates and Topics**
- **24 September, 2021:** OT setup, instrumentation, sterilization
- **01 October, 2021:** Laparoscopic Anatomy, Port position & entry diagnostic laparoscopy
- **08 October, 2021:** Energy Sources Ectopic Pregnancy
- **15 October, 2021:** Ovarian Surgery—Clinical Implications
- **22 October, 2021:** Endometriosis—Implants, Endometrioma & DIE
- **29 October, 2021:** Myectomy and Adenomyometry & fertility Tissue Retrieval and Morcellation
- **05 November, 2021:** Fertility Sparing Surgeries in Cancer

**Course Duration:**
- Live Interaction 2 hours Every Friday 5:00–7:00 pm

**For Queries, Contact**
- Dr. Damodar Rao: +91 950 333 4329
- Dr. Renu Mitr: +91 981 114 7217

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**Indian Fertility Society**

**Contact Details**
- www.indianfertilitiesociety.org
- info@indianfertilitiesociety.org
- facebook.com/indianfertilitiesociety
17th Annual Conference of Indian Fertility Society

**Fertivision 2021**

New Delhi | India

Workshops: 4 & 5 December, 2021
Conference: 10-12 December, 2021

Theme: “Challenges and Innovations in ART”

Organising Chairman: Dr Sudha Prasad
Organising Secretary: Dr Neena Malhotra
Scientific Chair: Dr K.D. Nayar

**ESTEEMED INTERNATIONAL FACULTY**

- Roberto Valiarelli (Italy)
- Antonios Makrigiannakis (Greece)
- Baris Ata (Turkey)
- Ben Mol (Australia)
- Danilo Cimadomo (Italy)
- Denny Sakkas (USA)
- Rachel Chin (Malaysia)
- Steven Fleming (Australia)
- Sesh Kamal Sunkara (UK)
- Peter Humaidan (Denmark)
- Peter Humaidan (Denmark)
- Rachel Chin (Malaysia)
- Steven Fleming (Australia)
- Sesh Kamal Sunkara (UK)

**12 PRE-CONFERENCE WORKSHOPS**

1. PGT (Embryo Biopsy and Applied Genetics) Hands On
   - INR 20000

2. Andrology and Semenology
   - INR 1500

3. Managing ART Pregnancy
   - INR 1500

4. Mastering the ART of Ovarian Stimulation in IVF
   - INR 1500

5. Challenges in OPU and ET
   - INR 1500

6. Laparoscopy in Endometriosis
   - INR 1500

7. Hysteroscopy - In ART
   - INR 1500

8. Regenerative Medicine in ART
   - INR 1500

9. Research Methodology in Reproductive Medicine
   - INR 1500

10. Ultrasound in Infertility and ART
    - INR 1500

11. Setting and Maintenance of ART Lab
    - INR 1500

12. Counselling in Infertility and ART
    - INR 1500

For More Information, visit
www.fertivision2021.com

**Conference Registration Details**

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<th>Category</th>
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<td>INR 2000</td>
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</tbody>
</table>

For More Information Call Mr Vikas Sharma
M: +91-9560493999 | Email: fertivisiondelhi@gmail.com