# Assessment of Post-Thaw Human Sperm Motility for Oligoasthenozoospermic Patients by Comparing Swim-up and Density Gradient Method.

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**Abstract**

The separation of motile spermatozoa is essential when clinical Assistive Reproductive Technologies (ART) began a quarter-century ago. As the indications for ART shifted from purely gynaecological to andrological over time, andrological research was compelled to better understand the physiology of male germ cells and develop more sophisticated techniques to distinguish functional spermatozoa from those that are immotile, have poor morphology, or are unable to fertilize the oocyte. Separation strategies based on different principles such as migration, filtration, or density gradient centrifugation evolved initially, starting with the simple washing of spermatozoa. The main purpose of this research is the assessment Of Post Thaw Human Sperm Motility for Oligoasthenozoospermia Patients. For this purpose, the motility of the sperm was calculated using two methods common in sperm preparation in ART – the Swim up and Density Gradient Methods. It was found that in the case of the density gradient method, total motility was 18.5 % with a confidence interval of 12.7 – 24.4. Post-thaw motility was 11.3% with a confidence interval of 6.1-16.5, post-thaw motility after 20 minutes was 6.5% with a CI of 3.1-9.9, and post-thaw motility after 40 minutes was 3.2% with a CI of 0.3 -6.1. Comparatively, in the swim-up method, the total motility % was 18 % with a CI of 11.9 – 24.1, post-thaw motility was 8.3% with a CI of 4.8 – 11.8, post-thaw motility after 20 minutes was 4.3 with a CI of 2.6 – 6.0, and the post-thaw motility after 40 minutes was 2.5% with a CI of 1.1 – 3.9. Though the observations showed that the density gradient method was slightly superior to the swim-up method with regards to the number of processed motile sperms from the preparation, the statistical analysis of our samples showed that there was no real significance between the two methods.

## 1. Introduction

Assistive Reproductive Technologies (ART) have shown exponential growth in the past two decades in sperm preparation methods (1). Multiple complex methods have been developed in the recent past. In addition to a high success rate, the ideal sperm preparation method should also be cost-effective, and be able to process a large volume of ejaculate (2). It is a common technique in ART to freeze the spermatozoa before the procedure (3). Since there is a choice of methods available for sperm preparation, we decided to compare the two methods: density gradient and swim-up method with regards to the post-thaw motility in Oligoasthenozoospermic patients.

## 2. Aim of the Study

The density gradient and swim-up methods are the two common methods used for sperm preparation. This study aimed to compare the post-thaw motility percentage between the density gradient method and swim-up method for Oligoasthenozoospermic patients.

## 3. Materials And Methods

Semen specimens from 10 men were taken for analysis. After 48-72 hours of abstinence, a sample from the subjects was collected by masturbation at the clinical laboratory. Semen analysis was performed on each sample after liquefaction. The subjects were explained about the study and written consent was obtained from them. Semen analysis was carried out as per WHO guidelines [18].
Source of sample: Sumathi fertility center, Madurai.
Inclusion criteria: 1) Sperm concentration less than 15million/ml
2) Sperm motility (progressive motility and non-progressive motility) less than 40%

## 4. Swim-Up Method

Ø After the semen has liquefied (usually 30 minutes

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at 37°C), 1-mL aliquot of semen are placed in 5-mL labeled tubes (Falcon TUBE) and gently overlaid with 2mL of medium (Quinn's Advantage/HEPES/human albumin):

Ø ALLOW TO CENTRIFUGE 1800rpm for 10 minutes. After centrifugation discards the supernatant solution without disturbing the pellet.

Ø Add 5ml of flushing media mix with pellet gently; centrifuge 1500rpm for 10 minutes discard the supernatant solution without disturbing the pellet.

Ø Add 2ml of IVF media and mix with pellet gently, centrifuge 1200rpm for 10 minutes discard the supernatant solution without disturbing the pellet.

Carefully add 0.5ml IVF media, Incubate tubes at 37°C or 45-60 minutes to allow progressively motile sperm to swim into the overlaid medium. Finally, check count and motility under a microscope.

## 5. Density Gradient Method

Prepare 80/40 gradients in a 14ml tube, carefully overlay liquefied semen sample, and centrifuge 2000rpm for 20 minutes. Carefully remove the gradients without disturbing the pellet.

Mix the pellet with a 5ml flushing media centrifuge at 1500rpm for 10 minutes. Carefully remove the supernatant solution without disturbing the pellet.

Mix the pellet with IVF media 2ml centrifuge 1200rpm or 8 minutes and discard the supernatant solution. Carefully add 0.5ml IVF media, Incubate tubes at 37°C or 45-60 minutes to allow progressively motile sperm to swim into the overlaid medium. Finally, check count and motility under a microscope.

## 6. Cryopreservation Protocol

1ml sperm freezing media (glycerol cryoprotectant) was added drop by drop to the post-wash semen sample (to prevent osmotic shock) now the sample was transferred to the cryovials. The sample was subjected to sequential cooling first at room temperature for 10 minutes, then in the refrigerator (4 degrees Celsius) for 10 minutes. Following this, the samples were frozen by static vapors cooling and then plunged into liquid nitrogen (-196 C).

## 7. Thawing Protocol

After cryopreservation, the cryovials were removed from liquid nitrogen and placed at room temperature. As the sample is subjected to a sudden change of temperature, small water droplets start to condense on the outer surface of the cryovials. This phenomenon is called sweating.

After this, the entire sample was taken and added to an equal amount of sperm wash media allow to centrifuge for 5 minutes at 1500rpm.supernatant was discarded and the pellet was layered with IVF media. The supernatant solutions were aspirated and checked the motility with the help of a makler chamber.

In both methods, samples were tested for post-thaw motility, post-thaw motility after 20 minutes, and post-thaw motility after 40 minutes after centrifugation.

## 8. Statistical Analysis

Statistical analysis was applied using the Statistical Package for Social Sciences (SPSS; version 26.00). The data analysis was done using an independent sample t-test for tables with the mean and 95% Confidence Interval of the Difference to compare the Density Gradient Method and Swim-up Method. P-value < 0.05 was used as a level of statistical significance.

## 9. Results

The results obtained from both methods are compared as given in Table 1. The parameters for comparison used were total motility percentage, post-thaw motility percentage, post-thaw motility after 20 minutes percentage, and post-thaw motility after 40 minutes percentage.

In the case of the density gradient method, total motility was 18.5 % with a confidence interval of 12.7 – 24.4. Post-thaw motility was 11.3% with a confidence interval of 6.1-16.5, post-thaw motility after 20 minutes was 6.5% with a CI of 3.1-9.9, and post-thaw motility after 40 minutes was 3.2% with a CI of 0.3 -6.1.

Comparatively, in the swim-up method, the total motility % was 18 % with a CI of 11.9 – 24.1, post-thaw motility was 8.3% with a CI of 4.8 – 11.8, post-thaw motility after 20 minutes was 4.3 with a CI of 2.6 – 6.0, and the post-thaw motility after 40 minutes was 2.5% with a CI of 1.1 – 3.9.

Statistical analysis of the samples on all parameters showed that there was no significant difference between the two groups regarding post-thaw motility. The comparison between the two groups is shown in figure 1.

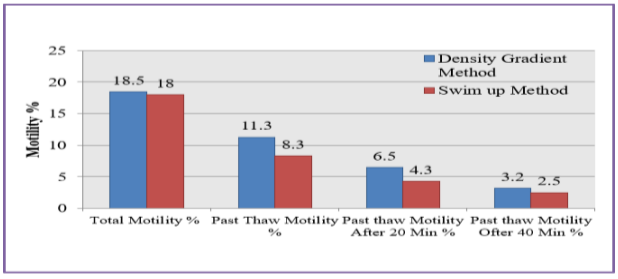


*Fig 1*

| Table-1 | | | |
|---|---|---|---|
| Parameter | Density Gradient Method | Swim up Method | P-Value |
| Total Motility % | 18.5 (12.7 – 24.4) | 18.0 (11.9 – 24.1) | .895 NS |
| Post Thaw Motility % | 11.3 (6.1 – 16.5) | 8.3 (4.8 – 11.8) | .297 NS |
| Post thaw Motility After 20 Min % | | 4.3 (2.6 – 6.0) | .209 NS |
| Post thaw Motility After 40 Min % | | 2.5 (1.1 – 3.9 ) | .634 NS |

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## 10. Discussion

The success of ART is known to be influenced by sperm factors. Although the World Health Organization's (WHO) reference values for sperm analysis are widely used to determine sperm quality, predictive sperm parameters and threshold values for successful ART remain disputed [1]. The number of inseminated sperm after a wash is thought to be a significant predictive factor [2].

Cryopreservation of spermatozoa is a common method for preserving male fertility. During semen banking, human spermatozoa are subjected to a wide range of cooling and warming speeds. They are less susceptible to cellular damage caused by rapid preliminary cooling, possibly due to increased membrane fluidity caused by the large concentration of unsaturated fatty acids in the cell's lipid bilayer. If the freezing-thawing technique is modified, the procedure may not harm human sperm function, notably motility.

Cryopreservation of sperm is a tried-and-true method of sperm banking. Semen samples that have been thawed can be used for artificial insemination or assisted reproductive methods in the future. Spermatozoa are reported to be less vulnerable to cryopreservation harm than other cell types due to the high fluidity of the membranes and lower water content (about 50 percent). Despite this, cryopreservation has the potential to alter the shape and function of sperm [3]. The production of intracellular and extracellular ice crystals, cellular dehydration, and osmotic shock have all been observed to occur during the freezing and thawing of human spermatozoa owing to heat shock [4]. The intracellular ice crystals that grow as a result of this process may penetrate membranes and disrupt organelle function. Cell survival may be harmed as a result of this. A sluggish cooling rate, on the other hand, controls the efflux of water from the internal to the exterior environment, raising the concentration of solutes and the osmotic pressure inside the cell. Dehydration causes cell volume alterations, as well as toxic damage from the high solute concentration [5]. Cryoinjury can happen during the freezing process as well as when the ice melts or is recrystallized during the thawing process [5].

Damage to the mitochondrial membrane has been blamed for the spermatozoa's decreased motility. To promote motility, ATP produced by oxidative phosphorylation in the inner mitochondrial membrane is transported to microtubules [6]. As a result, a decrease in motility could be explained by a decrease in mitochondrial activity [7]. Apart from investigating the impacts of freezing and thawing, this research demonstrates the impact of the post-thaw interval on the motility of human spermatozoa, extending the insemination window duration.

Even though motility has little to do with fertilizing potential, it is one of the most critical elements impacting sperm quality [8]. The motility of sperms is reported to diminish by 50% after the freeze-thaw cycle, according to Keel and Webster, 1993. After cryopreservation, however, there is a lot of heterogeneity in the susceptibility of individual donor sperms [9]. After reviewing the relevant literature, it was discovered that the majority of investigations focused on the effects of cryopreservation on vitality, morphology, and motility. There were also a few studies that compared the efficacy of different cryopreservation procedures.

According to the majority of methods used around the world, thawing is done for 5 minutes, then incubation for 10–15 minutes at 37 °C before insemination, [10]. Instead of blindly inseminating the semen sample 20 minutes after thawing, this study shows that it is possible to safely incubate the material until about 40 minutes after washing, analyze the motility, and then inseminated. This allows for the selection of the best possible samples (with proven motility), which is especially useful in the event of donor inseminations. Though previous studies have shown varied results regarding the method chosen, and the Thawing time, the current research finds that there is a significant difference does not exist between the two methods of Density Gradient and the Swim-up method used in sperm preprocessing. The research, is predominantly laboratory-based with limited samples, however before endorsing the approach for clinical usage, more studies along these lines in terms of pregnancy in ART cycles or fertilization rate in IVF/ICSI cycles can be carried out in the future.

## 11. Conclusion

ART is a rapidly developing field with complex mechanisms developed for sperm preparation. However, the newer methods must have a higher success rate and less contaminant in the process of preparing all the while being cost-effective also. In this study, we compared the two most familiar and widely used methods of sperm preparation, i.e., the density gradient method, and the swim-up method.

The main parameter for comparison we used in the study is sperm motility, i.e., total motility and post-thaw motility. Though our observations showed that the density gradient method was slightly superior to the swim-up method with regards to the number of processed motile sperms from the preparation, the statistical analysis of our samples showed that there was no real significance between the two methods.

Previous studies on the same topic have shown complex results where a study showed the superiority of the density gradient method (1), superiority of the swim-up method (4), and no significant difference between various sperm methods (5). This study shows that there is no significant difference between the two methods. However, we would like to point out that the challenges we faced in this study were: 1. Sample size was small, 2. The number of parameters for comparison was minimal.

There have been a smaller number of studies

reported from the South Asian community with regards to sperm preparation techniques and even lesser from the southern part of India. We welcome the research community to explore more on this topic to add to the existing evidence.

## 12. Ethical Clearance

Institutional ethics committee, Sri Balaji Dental College and Hospital, Committee registered SBDCH/IEC/02/2017/09.

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Conflict of Interest: Nil

## 13. Bibliography

[1] IltemirDuvan C, Berker B, Bayrak O, Aydos K, OzturkTurhan N, Satiroglu H. Comparison of semen parameters between pregnant and non-pregnant couples with male factor infertility during intrauterine insemination. *Turk J Med Sci.* 2009;39: 531–6.
[2] Kılıcdag EB, Bagis T, Haydardedeoglu B, Tarim E, Aslan E, Erkanli S, et al. The Prognostic Factors that Could be Effect Pregnancy Rates in Intra Uterine Insemination (IUI) Cycles. *TJOD.* 2005;2: 223–8.
[3] Watson P.F. The causes of reduced fertility with cryopreserved semen. *Animal Reprod Sci.* 2000;60–61: 481–492.
[4] Stanic P., Tandara M., Sonicki Z., Simunic V., Radakovic B., Suchanek E. Comparison of protective media and freezing techniques for cryopreservation of human semen. *Eur J ObstetGynecolReprod Biol.* 2000;91: 65–70.
[5] Said T.M., Gaglani A., Agarwal A. Implication of apoptosis in sperm cryoinjury. *Reprod Biomed Online.* 2010;21: 456–462.
[6] Zamboni L. The ultrastructural pathology of the spermatozoon is a cause of infertility. The role of electron microscopy in the evaluation of semen quality. *Fertil Steril.* 1987; 48: 711–734.
[7] O'Connell M., McClure N., Lewis S.E.M. The effects of cryopreservation on sperm morphology, motility, and mitochondrial function. *Hum Reprod.* 2002; 17: 704–709.
[8] Cross N.L., Hanks S.E. Ëffects of cryopreservation on sperm acrosomes. *Hum Reprod.* 1991;6: 1279–1283.
[9] Keel B.A., Webster B.W., Roberts D.K. Semen cryopreservation methodology, and results. In: Barratt C.L.R., Cooke I.D., editors. *Donor insemination.* Cambridge University Press; Cambridge, UK: 1993. pp. 71–96.
[10] Talwar P. Semen banking and cryobiology. *Int J InfertilFetal Med.* 2011; 2: 55–56.
11. Allamaneni SSR, Agarwal A, Rama S, Ranganathan P, Sharma RK. Comparative study on density gradients and swim-up preparation techniques utilizing neat and cryopreserved spermatozoa. Asian J Androl. 2005 Mar; 7(1): 86–92.
12. Mortimer D. Sperm preparation techniques and iatrogenic failures of in-vitro fertilization. Human Reproduction. 1991 Feb; 6(2): 173–6.
13. Yogev L. Pre-freezing sperm preparation does not impair thawed spermatozoa binding to the zona pellucida. Human Reproduction. 1999 Jan 1; 14(1): 114–7.
14. Chan C-C, Chen I-C, Liu J-Y, Huang Y-C, Wu G-J. Comparison of nitric oxide production motion characteristics of sperm after cryopreserved in three different preparations. Archives of Andrology. 2004 Jan; 50(1): 1–3.
15. Hammadeh M, Kühnen A, Amer A, Rosenbaum P, Schmidt W. Comparison of sperm preparation methods: effect on chromatin and morphology recovery rates and their consequences on the clinical outcome after in vitro fertilization-embryo transfer. International journal of andrology. 2001;24(6): 360–8.
16 Sanger WG, Olson JH, Sherman JK.semen cryobanking for men with cancer-criteria change .fertility sterility 1992: 58: 1024-1027.
17 Cryopreservation of sperms indications, methods and results. J Urol 2003,170: 1079-1084
18. Lu G, Hu S, Zhang Y, Chen J, Yuan Y, Gong X, Zhang Y. Analysis of Influencing Factors of Psychological Intervention on International Students in China after COVID-19: Hainan Province, China. American Journal of Health Behavior. 2022;46(6):606-17. https://doi.org/10.5993/AJHB.46.6.3