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Effect of Semen Freezing and Thawing on Sperm Survival and Motility Rate: A Comparative Analysis

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ABSTRACT

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Objective: To compare the rate of sperm survival and motility through semen freezing and thawing during infertility treatment. **Methodology**: In this bidirectional observational study, we enrolled 31 patients who underwent semen analysis for infertility treatment at the Institute of Reproduction and Child Cares & IRCC IVF Centre, Panchkula, Haryana, from June 2020 to December 2020. Out of these patients, 21 (67.74 %) were considered for semen freezing and thawing. For the rest of the ten patients (32.25 %), sperm count and motility were not good, and we excluded them from this study. Semen freezing based upon sperm count and motility were done. We did semen thawing after two weeks of semen freezing and recorded the sperm survival and motility. **Results:** Post thaw sperm survival rate and motility was 37.66% compare to pre-cryopreservation (61.82%). The observed rate of sperm motility declining was 24.16 % after cryopreservation/freezing. **Conclusion:** The present study results concluded that sperm's cryopreservation results in a decrease in sperm motility. There is a need of finding more accurate and reliable methods to freeze and thaw semen.

Keywords: Semen Analysis, Semen Freezing, Freezing Media, Semen Thawing, Sperm Motility and Storage in Liquid Nitrogen.

I. INTRODUCTION

The cryopreservation or freezing of spermatozoa was introduced in the 1960s as a fertility preservation mechanism (**1**). Freezing of human semen is an essential procedure for fertility preservation of cancer patient and patients undergoing infertility treatment as a backup purpose. Even some fertile couples can experience difficulties in conceiving due to geographical or medical challenges. A general deterioration of male reproductive health over time is also an essential indication for sperm banking **(2, 3**). Intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) have completely advanced the

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male-factor infertility treatment. As a result, sperm freezing or cryopreservation has become a successful treatment option (**4**). Cryopreservation is a procedure where cells, tissues or other substances vulnerable to chemical damage over time are preserved by cooling at -196^{0}C temperature. In 1957, the cryopreservation of tissues began with the freezing of fowl sperms (**5**). Cryopreservation was applied to humans in the mid-1950s (**6**). In the present scenario, cryopreservation is the most valuable process used to preserve sperm in men undergoing gonadotoxic treatment such as radiotherapies or chemotherapies (**7, 8**), which may lead to testicular failure or ejaculatory dysfunction. In addition to these, sperm cryopreservation is also offered in some non-malignant disorders, such as autoimmune diseases and diabetes (**9**), requiring treatments that may affect reproductive functions.

In the beginning, lowering the spermatozoa temperatures were observed by Lazaro Spallanzani in 1776(**10**). He observed that snow slowed the motions of sperms considerably, but sperms reached their previous motility when re-warmed.

Before 1949, many scientists recorded that sperm could survive freezing, but out of them, only a few could successfully fertilize the oocyte afterwards. In 1949, Polge et al. laid the foundation for cryopreservation of human sperm when they found a glycerol method to protect sperm injury during freezing. The first human pregnancy with frozen spermatozoa was successfully achieved in 1953 by Dr. Jerome K Sherman with other refined methodologies **(11)**. Sperm motility, plasma membrane functionality, acrosome integrity and viability in post-thaw typically decrease in contrast to the pre-freeze state**(12)**. Nijs et al. reported that the percentage of motile spermatozoa decreased from 50.6% to 30.3% after cryopreservation **(13)**. The present study aims to determine the observational differences and carry out a comparative study of freezing and thawing semen regarding sperm survival and motility during infertility treatment.

II. METHODOLOGY

Study design:

This bidirectional study span was seven months (6–months for laboratory work and 1–month for documentation and statistical analysis) from June 2020 to December 2020.

Patients:

A total of 31 Patients visited for semen analysis at the Institute of Reproduction and Child Cares & IRCC IVF Centre, Panchkula, Haryana, were included in this study. Out of 31 patients undergoing semen analysis, 21(67.74 %) were considered for semen freezing and thawing in this study. Twenty-one patients' sperm count and sperm motility before cryopreservation and two weeks of semen thawing were recorded **(Table 1).**

III. METHODS

All patients' routine semen analysis was done using the WHO manual of semen examination 2010(14). The patients with suitable sperm count and motility were enrolled in this study, and Semen freezing based upon sperm count and motility were done. We have used a single semen freezing protocol with a single semen freezing medium [**FertiPro, Belgium**] for all semen samples. We did semen thawing after two weeks of semen freezing and recorded the sperm survival and motility. The patients' data were categorized into two groups; pre cryopreservation data and post-thaw data. The consents of all the patients were taken for this study.

INCLUSION CRITERIA:

Patients with the following characteristics were included:

1. Patients with good sperm motility

2. Patient with good sperm count

EXCLUSION CRITERIA:

The patients with severe oligozoospermia, azoospermia and necrozoospermia were excluded.

SEMEN CRYOPRESERVATION:

In a sterile container, the semen sample was collected and kept at 37⁰C for liquefaction. After liquefaction, the routine semen examination was done according to the world health organization' manual**(14)**. Sperm concentration, motility and other parameters were recorded. Before use, 0.7 ml of semen freezing medium was kept at 37⁰C to warming and equilibration. 1 ml semen sample was cryopreserved by adding equilibrated semen freezing medium drop by drop within 10 minutes with continuous gentle shaking at 37⁰C. This suspension was loaded into the pre-labeled cryovial, and vapor freezing was done using LN2 for at least 10 minutes. After 10 minutes, the vials were dipped in LN2 (-196⁰C) and shifted in a proper canister till the thawing procedure. The details of the cryopreserved vial (location and storage) were recorded in the freezing record book.

SEMEN THAWING:

The cryopreserved vial was taken out from the cryocane of the LN2 storage cylinder and put at room temperature water for 5 minutes, and then kept in the incubator at 37⁰C for 30 – 60 minutes for better sperm motility. Thawing is a rapid, manual, re-hydrating process executed with accuracy; otherwise, even an ideally frozen sample resulted in the feeble recovery of sperm post-thaw. After equilibration of the thawed sample, routine semen analysis was done. The sperm survival and motility were recorded.

STATISTICAL ANALYSIS:

Data analyses were done using the SPSS version 20 (IBM SPSS Statics for Windows, Version 20.0. Armonk, NY: IBM Corp.). T-test was employed to saw the difference between mean values in the two groups. *P<0.05* was considered statistically significant.

IV. RESULTS

In this pre cryopreservation and post-thaw sperm motility data of 21 patients **(table 1),** we analyzed those undergoing infertility treatments for seven months, June 2020 to December 2020. The respective average pre-cryopreservation and post-thawing sperm count was 108.66 million and 75.61 million. The average difference between these two was 33.04 million (30.40%). Like sperm count, the average pre-cryopreservation and post thawing sperm motility was 59.42% and 34.71%, respectively. The average difference between Pre-cryopreservation and post thawing sperm motility was 24.71% which is depicted as a declining sperm motility rate after cryopreservation/freezing. This difference in sperm count and sperm motility values was significant **(table 2)** and showed an overall decline in these parameters of semen.

Table 1 : General characteristics

S. no.	Patient ID	Semen volume in ml	CPAs in ml	Pre-cryopreservation		Post thawing		Difference in pre and post sperm count	Difference in pre and post motility %
				Sperm count	motility type a+ type b	Sperm count	motility type a+ type b		
1	0124	1.0 ml	0.7	95	88	66	45	29	43
2	0125	1.0 ml	0.7	28	15	19	3	9	12

3	0126	1.0 ml	0.7	40	35	24	15	16	20
4	0168	1.0 ml	0.7	200	95	140	55	60	40
5	0176	1 ml	0.7	65	55	45	40	20	15
6	0179	1 ml	0.7	160	65	112	45	48	20
7	0178	1 ml	0.7	160	75	112	55	48	20
8	0179	1 ml	0.7	110	55	77	45	33	10
9	0185	1 ml	0.7	60	60	42	35	18	25
10	0187	1 ml	0.7	26	20	18	3	8	17
11	0190	1 ml	0.7	50	35	35	35	15	0
12	0199	1 ml	0.7	220	55	154	35	66	20
13	0238	1 ml	0.7	90	60	63	40	27	20
14	0252	1 ml	0.7	162	58	113	45	49	13
15	0253	1 ml	0.7	105	90	73	45	32	45
16	0254	1 ml	0.7	180	95	126	75	54	20
17	0256	1 ml	0.7	127	50	88	5	39	45
18	0257	1 ml	0.7	70	65	49	30	21	35
19	0258	1 ml	0.7	115	65	80	35	35	30
20	0259	1 ml	0.7	124	67	86	16	38	51
21	0260	1 ml	0.7	95	45	66	27	29	18

Table 2 : t-test for difference in mean values in two groups

Variables	Pre-cryopreservation	Post thawing	Mean difference	t-value	p-value
Sperm count	108.67±55.92	75.62±39.44	33.04±14.94	2.213	**0.033***
motility (type a+ type b)	59.43±22.08	34.71±18.36	24.71±6.26	3.943	**0.000***

***p-value <0.05 is significant**

V. DISCUSSION

Cryopreservation of male gametes is a vital aspect of human fertility preservation. With the advancement in assisted reproductive technology, indication for sperm cryopreservation is expanding (**Fakhrildin 2013**). Sperm cryopreservation is a broadly used method to maintain and protect fertility during infertility and malignancy treatments **(15).** Further, sperm Cryopreservation epitomizes as the only preemptive measure for conserving fertility, enabling less infertile men to father their children **(16).**

In the present study, we have enrolled 31 patients for semen analysis during infertility treatment. Out of 31, only 21 patients were found eligible to be included in this study for semen freezing and thawing. We observed a low sperm survival rate and motility post thawing, lower than sperm motility pre cryopreservation (61.82%vs37.66%). Similarly, there was a decline of 24.16 % in sperm motility rate after cryopreservation/freezing. These results are in

concordance with previous studies **(10, 17, 18)**. A study by Fakhrildin revealed a considerable, highly significant decrease in sperm parameters, including sperm concentration, sperm motility, progressive motility, and normal sperm morphology compared to pre-cryopreservation**(17)**. Freeze-thawing caused a 37% reduction in typical morphological forms of sperm **(18).** The motility of sperms is found to decrease after cryopreservation **(10).** Sperm cryopreservation is a crucial factor of fertility management whose success determines the reproductive outcome of assisted reproduction technologies (ART). The use of suitable cryoprotectants and sperm selection technologies before and after cryopreservation seem to have the most significant impact on preventing DNA fragmentation, thus improving sperm cryo-survival rates **(19).** After cryopreservation/thaw, a significant decrease in sperm motility and viability was observed, along with sperm DNA fragmentation and DNA oxidative damage **(20).**

It was found that cryopreservation has deleterious effects on various parts of spermatozoa, especially on plasma lemma, acrosome and tail. Previous studies suggested several factors (like sudden temperature changes, ice formation and osmotic stress) during the cryopreservation that are reasons for poor sperm quality post-thaw **(1)**. Post-thaw light microscopic studies revealed a considerable reduction in average spermatozoa viability rate **(15)**. Age is also correlated with a deterioration of various semen parameters (volume, motility and morphology) except sperm concentration. A review by Kidd et al. reported a decrease in semen volume, sperm motility and normal sperm when a comparison was made among 30-year-old men and 50-year-old men **(3).** Cryopreservation has deleterious effects on various parts of spermatozoa, especially on plasma lemma, acrosomes and tails. Post-thaw light microscopic studies revealed a considerable reduction in average spermatozoa viability rate **(15)**. Previous studies suggested several factors (like sudden temperature changes, ice formation and osmotic stress) during the cryopreservation that are reasons for poor sperm quality post-thaw **(1)**.

However, cryopreservation should be offered to patients with several malignant or non-malignant diseases. A study found no statistically significant differences in sperm parameters when various diseased patients were studied. However, patients with diabetes showed poorer sperm counts **(9)**. It was proposed that by simulating conditions similar to the in vivo conditions for the post-thaw semen samples. After thawing, waiting for some time is crucial to confirm the motility and count parameters of sperm before inseminating the samples. Blind insemination immediately after thawing should be avoided **(10).**

VI. CONCLUSION

In summary, we found a considerable decline in sperm motility after cryopreservation and observed after thawing, sperm survival and motility was 37.66 %. In comparison to pre cryopreservation, sperm motility was 61.82 %. The reason for this may be the type of cryopreservents used. Age may be the other factor that can cause a reduction in semen parameters. Non-malignant diseases like diabetes can also be one of the reasons for the decline in semen parameters. However, cryopreservation should be offered to patients with several malignant or non-malignant diseases and fertility preservation. Further studies are required to confirm the present findings in large sample size, along with the determination of underlying causes of decrease in semen parameters after cryopreservation and after the thaw.

VII. ETHICAL ISSUE

The independent institutional ethical committee approved this bidirectional observational study of semen freezing and thawing regarding sperm survival and motility of IRCC Hospital, Panchkula, Haryana, India.

VIII. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Authors Contributions:

RD is responsible for designing the experiments; SS, PK associated with laboratory work; SKT responsible for the preparation of the manuscript; K.S. for clinical diagnosis of the patients; PP is responsible for preparation of the manuscript.

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