ISSN No:-2456-2165

Comparative Study of Sperm Deoxyribonucleic Acid (DNA) Fragmentation Index Rate to Observe the Best Method of Sperm Preparation

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Abstract:- Sperm DNA fragmentation index or Integrity test is the important tool for the male infertility. Now days there are many factors that involve the damaging of Sperm DNA. Our life style is responsible for the higher DNA integrity level of sperms. Damaged DNA is responsible for infertility, Impaired reproductive outcomes after any ART procedure, Increased risks for genetic/birth defects and rapid or continuous miscarriages.

- ***Objective:***

To observe the best method for sperm preparation that results the healthier transmission of genetic information through sperms.

- ***Material and Methods:***

Double Layer density gradient with Hepes buffered HTF medium, single layer density gradient with Hepes buffered HTF medium, Simple sperm wash with culture media contains Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 EU/ML, gentamicin sulphate 10 ug/ml, DNA fragmentation integrity kit.

- ***Results:***

50 semen samples were processed to check the sperm DNA integrity. Each semen sample was processed with all three methods. It was found that semen samples processed with Double Layer density gradient with Hepes buffered HTF medium having less Sperm DNA fragmentation index followed by the single layer density gradient with Hepes buffered HTF medium. The highest Sperm DNA fragmentation index was found with Simple sperm wash method, contains Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 EU/ML, gentamicin sulphate 10ug/ml.

Keywords:- *Endotoxin, HTF Medium, Human Serum Albumin, Male Infertility, Sperm DNA Integrity, Reactive Oxygen Species (ROS).*

I. INTRODUCTION

Sperm cell is the male gamete cell which penetrates, capacitate and fertilize the female gamete cell (Oocyte). The main aim of the male gamete cell is to transfer the genetic information/material. A healthy Sperm cell transfers the healthy DNA or Healthy genetic information [1]. Oxidative stress is responsible for the alteration of the sperm DNA fragmentation integrity [2]. There are many factors that are responsible for increased level of DNA fragmentation index e.g. Increased BMI, varicoceles, environmental toxins, infrequent ejaculation, infection of reproductive organs, poor diet, alcohol, pollution, sedentary life style increase the temp. of testicles[3].

The microscopic examination of the sperms is the best tool for the evaluation of the male infertility. Morphological abnormalities can be identified as a suspected increased level of sperm deoxyribonucleic acid fragmentation index. Higher DNA fragmentation index associated with decreased rates of fertilization, pregnancy, quality of embryos and high miscarriages rates [4].

The transmission of genetic information is depends upon the integrity of the Sperm DNA Fragmentation index should be less than 15 % referred as an excellent to good sperm DNA Fragmentation index or DNA integrity [5]. Due to limited capacity of DNA repair, reactive oxygen species attack the DNA damaged DNA affects the Motility, Rapid linear progression and morphology of the sperm [6].

II. LITERATURE REVIEW

A study conducted by Akira Komiya et. al. in 2014, in which 54 semen samples were processed Out of 21 (38.9%) were collected from the patients with idiopathic causes of infertility. The remaining 33 (61.1%) were from those with varicoceles patients. Twenty- seven (50%) were from the patients who reported chronic alcohol use to some extent. Ten (18.5%) samples were collected from smokers. In this study the mean Sperm DNA fragmentation integrity rate was evaluated in processed semen samples was 41.3%. Consumption of alcohol increased the Sperm DNA fragmentation integrity rate 49.6 ± 23.3%. The SDFI was not related to the causes of male infertility [7].

A Study conducted by F. Bronet et. al., in 2011 in which 154 embryos were checked/examined from 38 patients undergoing PGD cycles; 35.2% embryos were found to be as chromosomally normal. An increased DNA fragmentation index rate found after semen washing or preparation in 76% of the patients. The study concluded that there was no correlation in between DNA fragmentation and the quality rates in embryos [8].

A prospective study conducted by Vivian Berkenbroch Ramos et. al., in 2013-2014, in which 20 normospermic samples were processed for ART and Recovery rates were 25% for migration- sedimentation, 10.1% for swim-up from fresh semen and 4.5% for swim-up from washed. migration- sedimentation recovery rate was higher (P<0.01) than the two swim-up techniques. Total motility was statistically different (P<0.001), with 93.6% for migration- sedimentation, 91.2% for swim-up from fresh semen, and 77% for swim-up from washed. The conclusion of the study was found as migration-sedimentation technique is best one as compare to the other two mentioned techniques [9].

III. MATERIALS AND METHOD

50 semen samples were collected from the ART Clinics/Centres. Research was done in the Punjab, India. Three different types of washing techniques Double Layer density gradient with hepes buffered HTF medium, single layer density gradient with hepes buffered HTF medium, Simple sperm wash with culture media contains Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 eu/ml, gentamicin sulphate 10 ug/ml wereperformed.

- *Double Layer Density Gradientmethod:*

After the liquefaction, entire semen specimen was placed in a Round bottom tube. Semen analysis was performed on Makler's counting chamber. Medium was equilibrated for at least 2 hours before processing thesample.

Entire semen sample was added to the top of 90% and 45% gradient medium. Tube containing 90% and 45% gradient medium and semen sample was centrifuged at 1500rpm for 8 minutes. Supernatant was discarded and pallet was re-suspended with 2-3ml of sperm wash medium and centrifuged at 1500 rpm for 8 minutes. Again supernatant was discarded and pellet was layered with 0.7ml of culture medium. 0.5ml of culture medium was taken from the top of tube after 20 min. culture medium containing healthy sperms was stored in a separate sterile tube at 37°C till insemination. DNA Fragmentation test was performed after the washing of semen sample.

- *Single Layer Density Gradientmethod:*

After the liquefaction, raw semen sample was added to the top of Single layer density gradient medium and centrifuged at 1600 RPM for 8 minutes. Supernatant was discarded and pallet was re-suspended with 2-3ml of sperm wash medium and centrifuged at 1500 rpm for 8 minutes. Again supernatant was discarded and pellet was layered with 0.7ml of culture medium. 0.5ml of culture medium was taken from the top of tube after 20 min. culture medium containing healthy sperms was stored in a separate sterile tube at 37°C till insemination. DNA Fragmentation test was performed after the washing of semensample.

- *Simple Sperm Wash Method:*

After the liquefaction, raw semen sample was mixed with the equal amount of semen washing medium (HTF) and centrifuged at 1500 RPM for 8 minutes. Supernatant was discarded and again mixed with 1 ml of semen washing medium (HTF) and centrifuged at 1500 RPM for 8 minutes. Again Supernatant was discarded and layered with 0.5 ml of culture medium containing Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 eu/ml, Gentamicin Sulphate 10 ug/ml. 0.4 ml of culture medium was taken after 20 minutes and kept in a sterile tube at 37°C. DNA Fragmentation test was performed after the washing of semen sample.

IV. RESULTS

Total 50 normospermic semen samples were processed with all three semen processing methods (Double Layer density gradient with hepes buffered HTF medium, single layer density gradient with hepes buffered HTF medium, Simple sperm wash with culture media contains Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 EU/ML, Gentamicin Sulphate 10 ug/ml).

The mean DNA fragmentation index/integrity rate of Raw samples, processed with Double Layer density gradient, single layer density gradient, Simple sperm wash were found as 38.8% +- 1.5% , 09.8 % +- 1.5 %, 14.5 % +- 1.5 %, 26.5% +- 1.5 % respectively. Data of processed samples is described below in tabular form:

	Raw sample	Double Layer density gradient	single layer density gradient	Simple sperm wash
DNA fragmentation index/integrity rate in %	38.8%+- 1.5%	09.8 % +- 1.5 %,	14.5 % +- 1.5 %,	26.5% +- 1.5 %

Table 1

V. CONCLUSIONS

All three methods (1- Double Layer density gradient with Hepes buffered HTF medium 2- single layer density gradient with Hepes buffered HTF medium 3- Simple sperm wash with culture media contains Human serum albumin 5mg/ml, Endotoxin (LAL) <= 0.5 EU/ML, gentamicin sulphate 10 ug/ml) were compared to get the conclusion that which one is the best method to get the healthier sperms. Healthy sperms refer to the less % of fragmented DNA, Good motility, good rapid linear progression, normal morphology in the Prepared or processed semen samples.

The results of all three methods were analyzed and compiled and we found Double Layer density gradient with Hepes buffered HTF medium is the best method to get the healthy sperms with good/excellent DNA fragmentation index rate (comparatively). At the end we will recommend the Double Layer density gradient with Hepes buffered HTF medium for the insemination.

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