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Short Communication

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Can Utilization of Sperm DNA Fragmentation (SDA) Tests in Combination with Time Lapse Microscopy Help in Improving ICSI Implantation Rates in Unexplained Recurrent Implantation Failures Utilizing Double Stranded SDA as the Causative Factor for the same

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Abstract

Over 50% of intracytoplasmic sperm injection (ICSI) cycles don't display implantation. Hence laboratories make their maximum efforts to select the best embryos as far as implantation enhancement is concerned. Further utilization of available technologies like time lapse recording have been made in a large number of artificial reproductive technology (ART) centres. Various studies that utilize embryo kinetics have implicated that time when embryo cleavage may prove to be an important factor that determines the implantation potential of an embryo. With this variety of algorithms mathematic wise have been used to forecast which the best embryos are for transfer. But the efficacy of these might be influenced by multiple confounding factors. Thus work on biomarkers that can forecast good ART warrants newer embryo selection basis. Regarding conventional ICSI, typical standard routine semen analysis involving sperm concentration, motility and morphology does not predict the implantation percentages in an ICSI cycle. Once sperm DNA fragmentation (SDA) methods were inducted they appeared to hold promise in forecasting good ART success. Although certain studies utilizing various techniques like TUNEL. SCSA, SCD proved a relation existed between DNA damage and implantation rates in ICSI but the same was contradicted by others. With this it was thought that bias between evaluation of ejaculate and motile sperm picked up for ICSI, as is known regarding absence of positive association of sperm motility and DNA fragmentation. Thus study by Casanovas et.al., tried to find if there is any correlation of single stranded (ssSDA) and double stranded (dsSDA) sperm DNA damage that might forecast ICSI success and utilizing Neutral Comet Assays along with help of time lapse technology they found that double stranded sperm influenced delay in embryo formation as seen by embryo kinetics and thus interfere with implantation rates. Reproduction of these findings might help in getting a standard for getting best embryos selected in ICSI utilizing SDA and time lapse microscopy.

Keywords: Strand Displacement Amplification (SDA); terminal deoxynucleotidyl transfer dUTP nick nd Labeling (TUNEL); Sperm Chromatin Structure Assay (SCSA); Sperm Chromatin Dispersion (SCD); single stranded (ssSDA); double stranded (dsSDA); Artificial Reproductive Technology (ART)

Commonly utilized factors in semen analysis do not work out to be enough for examining for male infertility and cannot thus analyze the function of the spermatozoa. One aspect that has received importance is Sperm DNA damage that results in bad in vitro fertilization (IVF) results in view of disturbance in embryo formation. Interference with time taken to pregnancy, interference in cleavage time of the embryos, enhanced abortion incidence, escalates the pregnancy loss following IVF/ intracytoplasmic sperm injection (ICSI) along with enhanced congenital anomalies in the newborn [1].

Sperms carrying damaged DNA are still capable of fertilization of oocytes. It is not clear what are the methods utilized for preserving preimplantation embryos that possess damaged DNA. These embryos do possess some ability for DNA repair, it is not clear how much amount of DNA injury can these embryos bear [2]. On getting injured DNA genome gets within the embryonic genome ,and can result in replication ,transcription, and translation problems resulting in mutations occurring at a variety of embryonic phases .These might get conveyed to further generations and ultimately influencing the genome of their offspring's. Paternal genome remaining normal is

vital for both pregnancy onset and sustainance.

Initially utilization of sperm DNA fragmentation examination was queried. A systematic review with meta-analysis by Simon et al., corroborated that lot of proof was there regarding role of Sperm DNA damage on both IVF/ICSI results [3]. But Cissen et al., pointed that enough proof did not exist for utilization of sperm DNA fragmentation testing as a part of every case undergoing assisted reproductive technology (ART). Proof existed regarding prognosis for pregnancy and which option should be opted for, though more research was recommended [4].

Routinely processes of evaluation of embryo quality are determined by repeated examination of cell number, cell division, and fragmentation of cells, asymmetrical or symmetrical along with embryo getting compacted. Time lapse microscopy is employed for examination of morphokinetics of the embryo and thus given us novel embryo picking basis another topic important clinically [5-7].

Sperm DNA fragmentation (SDF) might be divided into single stranded (ss SDF) or double stranded (dsSDF). Various assays like terminal deoxynucleotidyl transfer dUTP nick end labelling (TUNEL) either by utilization of flow cytometry / fluorescence microscopy, ii) sperm chromatin structure assay (SCSA), iii) sperm chromatin dispersion (SCD) iv Comet assay are there for testing Sperm DNA damage. The alkaline Comet assay mainly finds ssSDF, while the neutral Comet assay can only test dsSDF. But certain workers presented that presence of an alkaline buffer within the Comet assay can find all single stranded and double stranded DNA damage, in addition to alkali-labile sites [8].

Recently Casanova et al., presented their work on double stranded DNA damage of the sperm, utilizing the neutral Comet assay displaying that > dsSDF markedly decreased embryo implantation rates in contrast to lesser dsSDF or > ss SDF as examined by time lapse microscopy simultaneously [9]. This group has been working on dsSDF in the last decade. Earlier they documented a correlation of dsSDF in cases of recurrent abortions where cause was not found especially in infertile couples where no female factor could be discerned [10,11]. There they tried to differentiate ss SDF and dsSDF assay reports in various clinical cohorts, conducting a study analyzing all the 5 assays detailed earlier and pointed that there was a part of the sperm nuclear matrix in the sustainance of DNA integrity. Many other works on ds breaks in somatic cells are present [12,13].

The design of this study was observational, double blind, prospective one where 196 embryos taken from 43 infertile couple were used [9]. Evaluation of the same semen sample that had been utilized for ICSI was done regarding ssSDF and dsSDF. Simultaneously they monitored embryo morphokinetics with the time lapse microscopy, getting the time of every embryo division. They observed that dsSDF might be the major kind of DNA damage having an influence on embryo kinetics. Their results displayed delayed embryo kinetics along with reduced implantation rates in case of > dsSDF present in the semen sample that had been utilized for ICSI. Conversely, > ss SDF had no significant influence on embryo kinetics as well as implantation rates. Variety of delayed effects on embryo kinetics was exhibited for various kinds of DNA damage. Every stage of embryo formation got delayed by dsSDF, though the maximum influence seen was in the 2nd polar body (PB) extrusion and the morula phases that had coincided with embryo DNA damage checkpoint activation, while ss SDF had its main influence on the pronucleus phase. But embryo kinetics later got back to normal at the latter parts of cleavage.

Though having a bigger sample size might have served best to understand the influence of DNA fragmentation when other factors that further effect like cases of history of DM along with smoking Moreover, including history of diabetes mellitus (DM) and smoking might have given insight with the knowledge that these things influence DNA fragmentation [14].

Concluding that dsSDF influenced both embryo formation and interfered with implantation, while not much effect on both embryo kinetics along with implantation rates was observed with ss SDF. Now if this gets reproduced by other group of workers needs to be seen. More work should also be carried out with reference to kind of DNA damage when there is presence of high paternal age, smoking and DM [15].

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