# CORRELATION BETWEEN TWO SPERM DNA FRAGMENTATION TESTS (TUNEL AND SCSA) AND EVALUATION OF TUNEL ASSAY INTER-LABS VARIABILITY

LE SAINT C.[1], VINGATARAMIN L.[1], ALIX S.[1], PHILLIPS S.[1-4], BELLOC S.[3], ZINI A.[1-4], KADOCH I.J.[1-2]

[1] CLINIQUE OVO, MONTREAL, QC, CANADA. [2] DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY, UNIVERSITY OF MONTREAL, QC, CANADA.

[3] LABORATOIRE EYLAU, PARIS. [4] MCGILL UROLOGY RESEARCH DEPARTMENT, MCGILL UNIVERSITY, MONTREAL, QC, CANADA.

Canadian Fertility and Andrology Society

62nd ANNUAL MEETING
22ND – 24TH SEPTEMBER 2016
Toronto, Ontario, Canada

## ABSTRACT

**Introduction:** Evaluation of sperm DNA damage (SDF) is becoming an important test to assess male infertility. Elevated SDF has been associated with poor reproductive outcomes. Several tests of sperm DNA damage have been developed, however, the most commonly used tests are the sperm chromatin structure assay (SCSA), the sperm chromatin dispersion test (SCD) and the terminal deoxynucleotidyl transferase deoxyuridine triphospahte (dUTP) nick and labeling (TUNEL) [1] . We have decided to adapt and validate the TUNEL assay as it can accurately measure both single and double-stranded DNA fragmentation [2-6]. We use a flow cytometer for the cell acquisition rather than an epifluorescent microscope.
Methods: We have validated a research use only kit APO-Direct (BD Pharmingen, CA, USA) by using a bench top flow cytometer, Accuri C6 (BD, Biosciences, MI, USA). Two stages of validation responding to the ICSH, ICCS were established. A pre-analytical stage to determine the parameters for sample processing and an analytical stage to validate the set-up of the flow cytometer's parameters. An inter-laboratory comparison was done using the same sample.

**Results:** The regression analysis depicting the relationship between the % DFI were obtained for each assay. In figure 1, the regression demonstrate the comparison between SCSA assay and TUNEL assay at ovo labo and the comparison between TUNEL assay at Eylau laboratory and TUNEL assay at ovo labo. The p value for both regression was lower than 0.0001. The correlation coefficient is $r^2 = 0.7$ and 0.8, respectively. We did the same comparison as given above but on freshly fixed sample. The results obtained were: SCSA vs TUNEL ovo labo, $p<0.0001$, $r^2=0.71$, TUNEL Eylau laboratory vs TUNEL ovo labo, $p<0.0001$, $r^2=0.75$ (Figure 2). Bland-Altman plot permit to calculate the bias between the three different assays. There is a standard deviation bias of 3.5 between ovo laboratory assay and SCSA assay , whereas there is a bias of 1.3 between Eylau TUNEL assay and ovo labo TUNEL assay.

**Conclusions:** We report a pre-analytical and analytical validation process to measuring sperm DNA fragmentation by TUNEL assay using a bench top flow cytometer.

## OBJECTIVE

The aim of the study was to develop and validate a method in measuring sperm DNA fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, using a flow cytometer.

## METHODS

Several patients were referred to the clinic ovo to have their semen samples examined by the andrology department. Seminal sample were obtained after 72H of abstinence. Following the complete liquefaction, semen specimen were evaluated for sperm concentration and viscosity. 1.25 Millions cells were fixed in paraformaldehyde (PAF) for 30 minutes. The PAF is then removed and replaced by ice cold ethanol. Fixed samples were stored at -20°C for a minimum of 24H and were analyzed within two weeks. Specific negative and positive controls were obtained. For the positive control, the fixed semen was degraded by DNAse. For the negative control, the enzyme Terminal deoxynucleotidyl transferase (TdT) is omitted during the incubation step. The staining method was prepared using APO-DIRECT kit (BD Pharmigen, CA, USA) by following its instruction. A bench top low cytometer was used for the cell acquisition and a minimum of 10,000 events were recorded. An inter-laboratory comparison was done between two different laboratories, Eylau laboratory (Paris, France) and Royal Victoria laboratory (Montreal, Quebec). The Eylau laboratory used TUNEL assay by In Situ Cell Death Detection Kit and Fluorescein (Roche diagnostics Coporation, Mannheim, Germany) with a flow cytometer (Beckman Coulter), whereas Royal Victoria laboratory used the SCSA method by flow cytometry.

## STATISTICS

Each sample was treated in duplicate. The comparison of the data obtained was performed using GraphPad Prism version 5.

## RESULTS

**Figure 1.** The correlation between SCSA, Eylau laboratory and ovo labo TUNEL assay ($p<0,0001$)

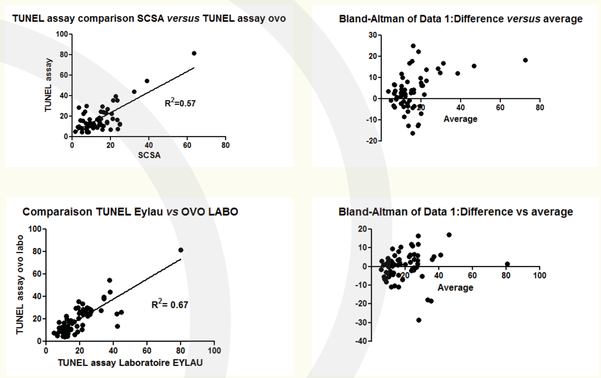
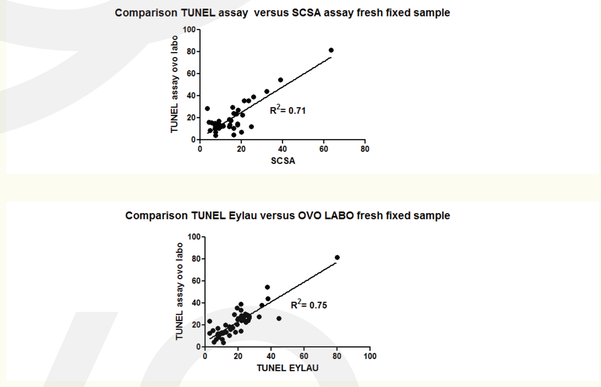



**Figure 2.** The correlation between SCSA, Eylau laboratory and ovo laboratory TUNEL assay (n= 32) with freshly fixed sample




Note that SCSA and TUNEL assay are two different method for detecting DNA fragmentation what explained the R square. Despite the difference, the results obtained give the same clinical decision.

## CONCLUSION

There is a significant bias between SCSA and TUNEL assay possibly due to the differences in these tests. The bias between ovo labo and Eylau lab is negligible. This correlation and comparison allows us to validate the results obtained with our assay and to be able to determine the appropriate process in handling sperm samples. After a complete liquefaction, samples were freshly fixed with 2% PAF and conserved at -20°C for a limit of 2 weeks. The purpose of this study was to propose the test to our patient having fertility problem. The cut off point is 16.8% set out by Sharma R et al., 2016 [7]. The high specificity of the TUNEL assay will be useful in correctly identifying infertile patients. This protocol is currently use in our laboratory to help patients who are having fertility problems.

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