P-2160

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

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AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

72TH ANNUAL MEETING
15th - 19th October, 2016
Salt Lake City, Utah

ABSTRACT

Introduction: Over the last decade, numerous studies has confirmed that by analyzing sperm DNA, the outcome of spontaneous pregnancy and assisted reproductive techniques can be better explained than traditional sperm parameters [6-7]. The most commonly performed tests to predict sperm DNA fragmentation (SDF) are sperm chromatin structure assay (SCSA) and the terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL). Here, at clinic ovo, we have adapted and validated on sperm sample, a research use only (RUO) kit to propose the assay to our patients.

Methods : We have two stages: a pre-analytical and an analytical validation [1-5]. The pre-analytical validation allows us to control the condition during the process of the sample, which consists of verifying the fixative reagents, the specimen storage, stability and transportation. As for the analytical validation, several parameters were calculated: linearity, repeatability, variability inter and intra assay and the background of the method. The TUNEL assay was compared with the SCSA assay for the same sample. An inter laboratory validation was also done with EYLAU laboratory (Paris, France) and Royal Victoria laboratory.

Results: When samples were fixed after nitrogen conservation versus when they were freshly fixed, there was a bias of 74.7% (Table 1). Upon this finding, we decided to fix the sample as quickly as possible with the reagent fixative. Once fixed, the samples are analyzed within two weeks. Patient data was determined over repeated time point to ascertain a natural variability. By assessing the intra assay variability (4.5%) and inter assay (7.8%), we found a bias of ≤ 5%. The background variability derived from the instrument was 10%. The assay linearity was also calculated by diluting a positive sample with a negative sample. The bias of the validation inter laboratory was 1.3%. R^2= 0.73 when comparing Eylau Laboratory (Paris) using TUNEL assay, and R^2= 0.57 comparing SCSA (McGill Urology Research Department, Montreal) (Figure 1).

Conclusions: We report a pre-analytical and an 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 adapt and validate a method for 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 andrology department at clinic ovo to obtain samples of seminal ejaculates. For the seminal samples to be eligible for testing, the patients has to practice 72H of abstinence. Once the concentration of the sample was calculated, 2.5 Millions cells were fixed with 2% PAF as soon as possible.

These sample allows us to developed the assay and do a pre analytical and analytical validation. To do so, we used a flow cytometer accuri C6 (BD Biosciences, MI, USA), and a KIT APO direct (BD Pharmigen, CA, USA). Samples were fixed and stayed at -20°C for a minimum of 24H and analyzed within two weeks.

Our method was compared with two laboratories, EYLAU Laboratory (Paris, France) and Royal Victoria Laboratory (Montreal, Quebec). EYLAU laboratory analyzed samples by TUNEL assay using In Situ cell Death Kit, fluorescein (Roche diagnostics Corporation, Mannheim, Germany) with a Beckman Coulter flow cytometer. Victoria Laboratory used the SCSA method coupled with a flow cytometer.

STATISTICS

Each sample was treated in duplicate. The average results, the coefficient of variation (CV) and the bias percentage was calculated using the software GraphPad Prism version 5.

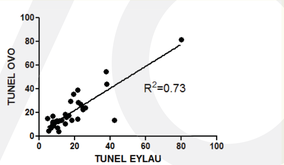
RESULTS

Table 1. Descriptive statistics of the samples stability: freshly fixed versus fixed after nitrogen storage (n=13).

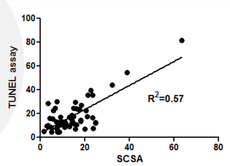
SAMPLES	% MEAN DFI A	% MEAN DFI B	% BIAS
FA-268	16,7	30,4	82,0
FA-271	8,6	12,4	44,2
FA-272	17,4	48,5	178,7
FA-273	34,2	60,2	76,0
FA-274	16,9	34,6	104,7
FA-275	7,3	21,1	189,0
FA-277	19,3	24,3	25,9
FA-279	21,1	29	37,4
FA-260	11,7	10,6	-9,4
FA-262	33	24,5	-25,8
FA-264	29,4	29,4	0,0
FA-265	17,4	22,9	31,6
FA-269	8,7	29,3	236,8
% MEAN BIAS			**74.7**

Mean DFI % A: Sample freshly fixed before analysis
Mean DFI % B: Sample store in nitrogen before fixation

Figure 1. Inter-labs validation



TUNEL assay comparison Eylau Laboratory versus ovo labo (n=32)



TUNEL assay comparison SCSA versus TUNEL assay ovo

CONCLUSION

We have discussed a detailed method of the development and validation in measuring sperm DNA fragmentation by TUNEL assay using a flow cytometer. This study allows us to standardize the parameters necessary to process patient's sample by following the agreement established by CLSI, ICSH and ICCS [1-5].

The purpose of this study was to propose the test to our patients having fertility problem. The cut off point is 16.8% set out by Rakesh S et al [6]. 2015. The high specifity 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 facing with fertility problems.

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