



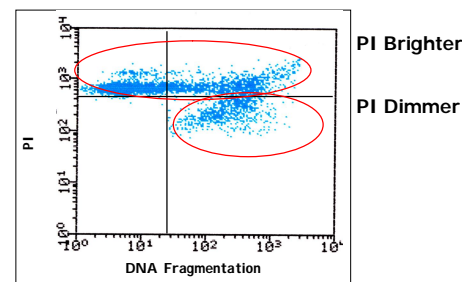
INTRODUCTION

Many Authors propose that tests determining sperm DNA Fragmentation (SDF) could provide predictive information to add to the routine semen parameters, the latter poorly predictive of the reproduction outcomes.

TUNEL/PI is an innovative flow cytometric version of the TUNEL assay for detection of SDF, and couples the detection of DNA breaks with the nuclear staining with propidium iodide (PI). TUNEL /PI has improved the accuracy (Cytometry part A, 2008) and the precision (J Androl, 2010) of the measures of SDF.

In addition, TUNEL/PI is able to reveal the occurrence of two sperm populations differently stained by PI and named PI brighter (PI br) and PI dimmer (PI dim) populations (Fig.1, Hum Reprod, 2008).

Fig.1



AIMS

To establish the mean values of SDF, as assessed by TUNEL/PI in a cohort of men with proven fertility.

To verify whether SDF is able to distinguish between sub/infertile and fertile men, independently from semen quality and age.

To determine the intra-individual variability of SDF .

METHODS

Recruitment of 67 fertile men (male partners of couples that conceived within one year from the day of the test) and of 90 sub/infertile subjects (male partners of infertile couples)

Matching between fertile and sub/infertile men, according to the age and the standard semen parameters (1 fertile:1-2 sub/infertile)

Recruitment of subjects undergoing TUNEL/PI test twice. The variability between the two measures was expressed as intra-individual coefficient of variation (CV).

RESULTS

Mean value and range of SDF in 67 men with proven fertility

FERTILE (n=67)	Mean±SD, (range)
SDF%	35.9±15.0, (12.0-65.5)
Sperm Count (x10 ⁶)	188.6±158.3, (5.1-780)
Concentration (x10 ⁶ /ml)	70.0±83.4, (3-520)
Progressive Motility (%)	47.0±16.3, (5-83)
Immotile sperm (%)	43.4.0±16.2, (13-91)
Normal Morphology (%)	9.2±6.0, (1-27)
Age (y)	36.2±5.0, (29-53)
Age Female Partner (y)	34.5±3.9, (26-53)

Fig.2 Comparison of SDF in fertile and sub/infertile men, after matching for semen quality and age

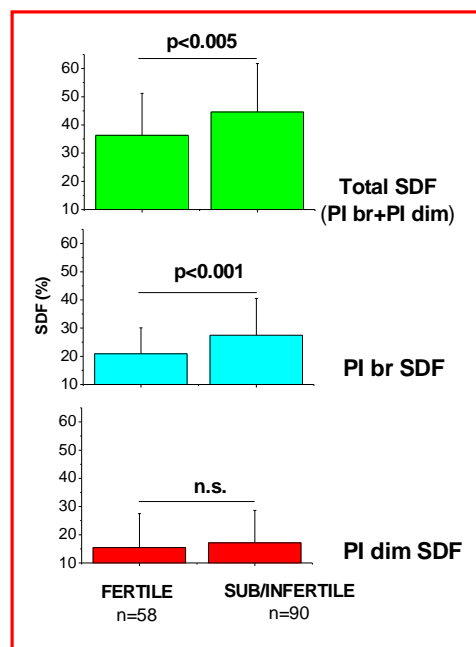
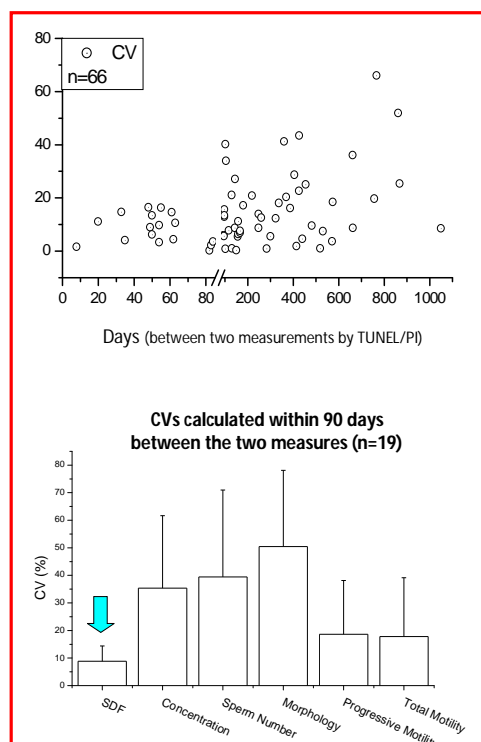


Fig.3 Intra-individual variability of SDF



CONCLUSIONS

The mean value of SDF in a cohort of 67 fertile subjects was: 36.4±14.8 %.

After matching for semen quality and age, sub/infertile men present greater amount of SDF than fertile ones. Such a difference is entirely due to the PI br SDF (Fig.2). Hence SDF, and in particular PI br SDF, is able to distinguish between fertile and sub/infertile subjects independently from age and semen quality. One limitation of this study is that we have no data on the female factor in the recruited infertile couples. It is possible that in a certain number of these couples, the infertility was due to a female factor and that the male partners was actually fertile. This would explain why the difference in SDF between the two groups is not so high.

Within three months, SDF results a very stable parameter intra-individually (Fig.3, mean CV=8.9±5.5%, n=19) and the most stable of any semen standard parameter. After three months, the intra-individual variability of SDF increases in about 50% of the tested patients (n=47).