

## The sperm chromatin structure assay (SCSA) as prognostic factor in IVF/ICSI program

Piotr Miciński<sup>1,2</sup>, Krzysztof Pawlicki<sup>3</sup>, Ewa Wielgus<sup>3</sup>, Michał Bochenek<sup>4</sup>,  
Iwona Tworkowska<sup>2</sup>

<sup>2</sup>Novomedica, Infertility Clinic, Mysłowice; <sup>3</sup>Department of Histology  
and Embryology, Silesian Medical University, Katowice; <sup>4</sup>National  
Research Institute of Animal Production, Kraków-Balice, Poland

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### SUMMARY

In this study 60 couples undergoing intracytoplasmic sperm injection (ICSI) procedures were enrolled. All men were classified into two groups regarding to the DNA fractionation index (DFI) threshold value: group I <15% and group II  $\geq$ 15%. In group I, median DFI was 4%, normal pre-implantation embryo development was observed and eleven pregnancies were achieved. In group II, median DFI was 23% and normal pre-implantation embryo development was also observed, but only two pregnancies were achieved. Our results suggest that the patients included in the assisted reproductive techniques (ART) should be diagnosed with the SCSA test and the DFI may be related to the outcome of fertilization process as well as to the number of transferred embryos and pregnancy. *Reproductive Biology* 2009, **9**, 1: 65-70.

**Key words:** spermatozoa, sperm chromatin, DNA fragmentation index, ICSI

<sup>1</sup>Corresponding author: Novomedica, ul. Bończyka 34, 41-400 Mysłowice, Poland, e-mail: micinski@ka.onet.pl

## INTRODUCTION

Several studies imply that the conventional sperm parameters (sperm concentration, motility and morphology) measured during a routine semen analysis are not helpful when intracytoplasmic sperm injection (ICSI) is used because they do not identify subtle defects in sperm chromatin architecture. Poor semen quality has been associated with an increase in the proportion of sperm with DNA fragmentation [3, 8]. Recently, the sperm chromatin structure assay (SCSA) has been recognized as an independent measure of the sperm quality that may have higher diagnostic and prognostic capabilities than standard sperm parameters for both *in vivo* and *in vitro* fertilization [1].

The SCSA is a rapid flow cytometry-based measurement of DNA that determines the percentage of spermatozoa with low, moderate or high DNA fragmentation expressed as the DNA fractionation index (DFI). The reproductive parameters affected by an increased presence of DNA abnormalities in ejaculated spermatozoa include fertilization, blastocyst development and pregnancy rates which are of great importance for assisted reproduction technologies [9]. The aims of this study were: 1/ to assess the sperm DNA fragmentation in candidates for IVF-ICSI, and 2/ to compare the embryo cleavage, implantation and pregnancy rates with the DFI values measured by the SCSA.

## MATERIALS AND METHODS

In this prospective study sixty couples (mean female age: 30.5±3.7, mean male age: 34.4±4.1) undergoing the ICSI procedures were enrolled. All men were classified into two groups regarding the DFI threshold value: <15 % (group I, n= 39) and ≥15% (group II, n= 21). Including criteria were: aged under 37 years, normal hormonal parameters, normal gynecological features and lack of infections. Excluding criteria were: woman aged above 37 years, abnormal gynecological and hormonal parameters. Permission was obtained from couples of both groups involved in the ICSI fertilization program. Pregnancies were confirmed by fetal heart rate (FHR) using USG.

Ovarian stimulation was achieved with conventional short or long protocols involving pituitary desensitization with GnRH analog (Decapeptyl, Ferring, Germany) and with ovarian stimulation with recombinant gonadotropin (Gonal, Serono, Switzerland), starting doses between 225 and 300 IU. When the follicles reached about 16-20 mm in diameter, the patients were given LH agonist (Ovitrelle, Serono, Switzerland) and 36 hours later the oocyte retrieval was carried out by vaginal USG-guided aspiration under general anesthesia.

Semen quality parameters - volume, pH, sperm concentration, motility and morphology - were determined according to the guidelines of WHO [11]. Intracytoplasmic sperm injection was performed as described previously [12]. At 16-18 hours after ICSI, the oocytes were assessed for fertilization (at pronuclear stage) and then 48 hours after oocyte retrieval the embryos were classified. The optimal number of 2-3 embryos (at 8 blastomeres stage) were transferred 72 hours (84%) or 96 hours (16%) after the ova retrieval.

The SCSA was performed using a flow cytometry as described for mammalian spermatozoa [5]. The fluorescence of green (515-530 nm) and red (>630 nm) bands was measured by DAKO Galaxy flow cytometer. Current statistical clinical thresholds had been established to <15% and  $\geq$ 15% DFI for normal and a decreased fertility potential, respectively. For statistical analysis, the non-parametric Mann-Whitney U test was used. Correlation coefficient was performed with Spearman's test. Data are presented as median and lower/upper quartiles. Statistical significance was set at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

In patients with DFI  $\geq$ 15% (group II), the observed percentage of normal spermatozoa and spermatozoa with progressive motility were significantly lower than in group with DFI in 0-15% range (group I; tab. 1). The number of fertilized oocytes was similar for both groups, but the number of unfertilized oocytes was higher in group II than in group I. Moreover, we found a positive correlation between DFI and unfertilized oocytes ( $p < 0.05$ ). In both groups, early embryo development did not differ and the mean number

Table 1. Selected parameters of patients undergoing ICSI procedure

Parameters	Group I			Group II		
	n = 39			n = 21		
	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile
DFI (%)	4.00	3.93	5.87	23.00*	18.44	24.75
Spermatozoa with progressive motility (%)	7	5	1	4	2	6
Morphologically normal spermatozoa (%)	69	59	73	59*	53	65
No. of oocytes	11	8	14	12	15	15.5
No. of infertilized oocytes	3	2	5	5*	3	7.5
No. of fertilized oocytes	5	3	6	5	3	6
No. of blastomeres	10	8	12	8	8	14
No. of transferred embryos	3	2	3	2*	2	3
No. of pregnancies	11			2*		

\*statistical significance  $p < 0.05$

of embryo blastomeres was similar. In group I, 3.0 (interquartile range 2-3) and in group II, 2.0 (interquartile range 2-3) embryos were transferred and differences between the two groups were statistically significant ( $p < 0.05$ ). In group I we noted eleven pregnancies, but in the group II only two pregnancies were achieved ( $p < 0.05$ ).

Our data showed that only two pregnancies were initiated when DFI was  $\geq 15\%$ , suggesting that sperm DNA damage has a good predictive value in cases of post-implantation embryo development failure. Sperm DNA integrity, cleavage rates, embryo quality and successful establishment of pregnancy following IVF cycles were reported in some studies [7, 10]. Agrawal and Said [2] proved that sperm DNA damage shows a negative

correlation with embryo quality following IVF and ICSI; however, it remains unclear whether spermatozoa with damaged DNA can impair the process of embryo development. The quality of sperm chromatin packing affects pregnancy rates after IVF. Filatow et al. [5] suggest that some mechanism may prevent an embryo from developing when the organization of genetic material in male germinal cells is abnormal. Spermatozoa with damaged chromatin can be morphologically normal and reveal the capability for penetrating the oocyte but they are incapable of initiating normal embryo development [4].

In clinical practice, especially with the usage of ICSI, treatment of male fertility still relies on diagnosis based on the light-microscope or computer-aided sperm analysis of sperm morphology and motility. Larson et al. [6] and Zini et al. [13] suggested that sperm chromatin is an independent measure of sperm quality that may have better diagnostic and prognostic capabilities required for an optimum fertility status than standard sperm parameters. The SCSA has become an important tool for assessing semen quality in the human andrology laboratory as well as in the context of assisted reproductive techniques (ARTs) used for treating infertile couples. Our results suggest that patients included in the ART should be diagnosed with the SCSA test and the DFI may be related to the outcome of the fertilization process as well as to the number of transferred embryos and pregnancy.

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