

INVESTIGATIONS ON THE QUANTITY OF DEOXYRIBONUCLEIC ACID (DNA) FRAGMENTED FROM NUCLEUS OF HUMAN SPERM CELL

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Abstract: Infertility is “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.”(Zegers-Hochschild F. et al., 2009). This paper presents an aspect of male infertility, the percent DNA fragmentation from sperm's head and it's clinical application. To obtain the percent of DNA fragmentation from sperm's head we used sperm chromatin dispersion test commercialized under Halosperm test name. One of the factors involved in male infertility is the percent of DNA fragmentation from sperm's head. The present study was made at Gynatal Clinic from Timisoara, on 25 male patients, who addressed to the Clinic for couple infertility between June 2015-May 2016. The female partner was tested and the couple's diagnostic was idiopathic infertility. After the female partner was tested and excluded as a cause for the couple infertility, the sperm count and the percent of DNA fragmentation from sperm's head were performed. These tests were made in order to achieve more information and to help the specialist to choose the adequate human assisted reproductive technique. After this study, we may conclude that the measurement of the percent of DNA fragmentation from sperm's head has practical relevance. Taking in consideration the results of the percent of DNA fragmentation from sperm's head test, the specialist can decide the most suitable human assisted reproductive technique in order to maximize the attempt.

Keywords: male infertility, Halosperm, ADN

INTRODUCTION

Investigating couple infertility leads to the conclusion that about 50% of the cases are because of male infertility.

To improve the diagnose and treatment of male fertility, new diagnosis methods have been developed, besides analysis of seminal liquid and specific initial investigation, to decide upon the initiation of assisted human reproduction (AHR) procedures.

New studies suggest that the degree of fragmentation of DNA can be an important predictor of low male fertility. Patients with a high percentage of DNA fragmentation have a significant lower fertile potential.

The Halosperm method is a test measuring the level of DNA fragmentation from spermatozoon; its superiority resides in the proper selection of the technique of assisted reproduction with no failures that delay the final goal.

The goal of this paper is to present data produced by the Halosperm test and to emphasise the improvement of the success rate in assisted human reproduction procedures due to the application of academic conclusions in practice.

The Halosperm tests were made on human sperm samples and analysis of the blades to determine the DNA degradation percentage and diagnose was carried out at the Gynatal Laboratory of Assisted Human Reproduction in Timisoara.

MATERIAL AND METHODS

The study was carried out on 25 patients of the Gynatal Clinics in Timisoara.

The sperm was sampled according to laboratory procedures (Levitas *et al.*, 2000; Checiu Delia, 2010). After sampling, the recipients were tightly closed and taken over by the personnel of the clinic.

The patients were not under antibiotic or other drug treatment and they did not take drugs, smoke tobacco or drink alcohol in excess.

The DNA fragmentation percentage was determined with the spermatozoon chromatin dispersion (SDC) method known as Halosperm G2.

Halosperm is an economic, simple and improved SDC test measuring the sensitivity of the sperm DNA to acid denaturation. Spermatozoon classification is made because DNA-fragmented spermatozoa do not have a dispersed DNA halo characteristic to intact DNA spermatozoa (Fernandez *et al.*, 2005).

If it is clear that the fragmented DNA causes infertility, then patients can be administered orally an antioxidant treatment two months before the intracytoplasmic sperm injection (ICSI). This treatment is simple, well tolerated, and it decreases the DNA fragmentation percentage in most cases. If the treatment is not effective, we need to make a testicular puncture and then ICSI because the DNA fragmentation percentage in the spermatozoa taken directly from the testicles is lower than that of the ejaculated spermatozoa (Tesarik *et al.*, 2006).

RESULTS AND DISCUSSION

We tested sperm DNA fragmentation on 25 patients, the criterion of inclusion was the specialist's recommendation and the couples had been diagnosed with either idiopathic sterility or male infertility (oligospermia).

Of the 25 patients, 16 were diagnosed with normal spermia (values of spermatozoa concentrations higher than 15 million/ml), while the other 9 were diagnosed with oligospermia (values of spermatozoa concentration lower or equal to 7 million/ml).

The limit recommended by the producer for the sperm DNA fragmentation percentage is 30%; higher values are considered pathological.

Of the 16 patients with normal spermia, 10 had a sperm DNA fragmentation percentage below 30%. The other 6 patients had a sperm DNA fragmentation percentage above 30%. After testing and result interpretation, we decided that 62.5% of the patients had to undergo intrauterine insemination (IUI) as a method of assisted human reproduction given that the male partner has to be excluded as a cause of couple infertility.

In the other 6 patients (37.5%), because the DNA fragmentation test pointed to a large number of DNA-fragmented sperm (it is well-known that DNA-fragmented sperm, though not preventing fecundation, produces low-quality embryos with low implantation potential), we recommended the couples *in vitro* fertilisation (IVF) using the ICSI method (Gardner, 2006; Gardner *et al.*, 2001).

The IVF recommendation is because of the female/male pathology. In the 6 studied couples with no female pathology, the initial recommendation would have been IUI. The result of the sperm DNA-fragmentation test was one more reason to switch to IVF, a more successful method than IVF.

Among the 9 patients with oligospermia, we found a DNA-fragmentation test value above 30% in 7 patients and a value below 30% in 2 patients.

The values of spermogrammes in the 9 selected patients ranged between 5 and 7 million/ml, which recommended them for IVF. DNA-fragmentation test in these patients aimed

at increasing the percentage of success and it represented a decision factor in approaching the necessary AHR method.

As for the 7 patients in which the test value was above 30%, the IVF recommendation was turned into an ICSI recommendation. In the other 2 patients, the test result was one more reason to stick to the initial IVF procedure.

Graphs from figure 1 shows that in patients with normal spermia, the chances of sperm DNA-fragmentation percentage to be below 30% are higher than in the patients with oligospermia.

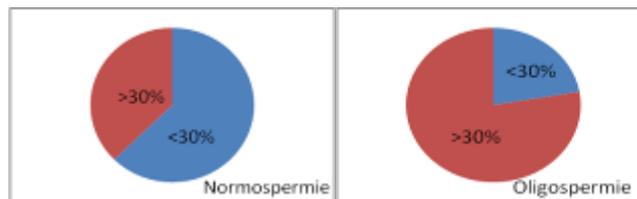


Figure 1. Sperm DNA-fragmentation percentage < 30% or >30% in the studied patients

To emphasise the efficacy of the DNA-fragmentation test, we compared the fecundation percentage of the sample of 9 patients with oligospermia (spermatogramme values between 5 and 7 million/ml) with a similar sample of 9 patients with oligospermia (spermatogramme values between 5 and 7 million/ml) with no female pathology and that were not tested for DNA fragmentation. In the control sample, in one case we identified the complete absence of fecundation despite the fact that the spermatogramme values pointed to a sufficient number of spermatozoa for fecundation to take place.

We made this comparison to see if there was one more reason to implement the DNA-fragmentation test on human sperm as a routine method in specialised laboratories.

However, statistically, these unique cases are insignificant; including the DNA-fragmentation test among routine analyses could have prevented the failure and, thus, reduce physical, physical and financial stress in the couple.

Based on these data, we recommend undergoing the DNA-fragmentation test to patients with oligospermia because it can make the difference between IVF and ICSI and avoid fertilisation failure. Of the 13 patients with DNA fragmentation below 30%, 7 underwent the ICSI, a method that would not have been recommended without test results (Table 1).

The ICSI method is the last option of human reproduction because it is aggressive to the gametes and because it involves supplementary costs for the patients.

Tabel 1.

Procedural recommendations after testing of the 25 patients

DNA Fragmentation			
<30%		>30%	
IUI	FIV	FIV	ICSI
10	2	6	7

Figure 2 shows normal spermia with lower degree of DNA fragmentation because there are 9 over 14 spermatozoa/field with a halo and 11 over 17 spermatozoa/field with a halo.

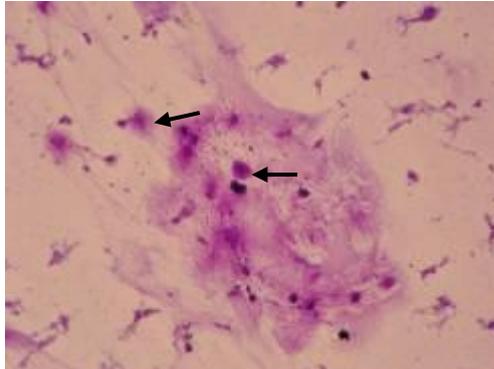


Figure 2. Halosperm <30% (x100) (original)

Figure 3 shows high fragmentation oligospermia. Though there are spermatozoa with a halo, it is a small one.

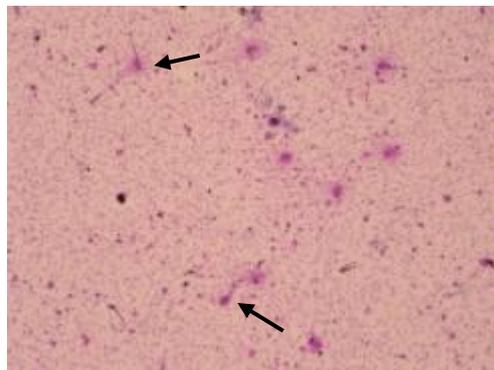


Figure 3. Halosperm >30% (x100) (original)

Figure 4 shows spermatozoa spread over the entire field pointing to a normal spermia with a low fragmentation degree.

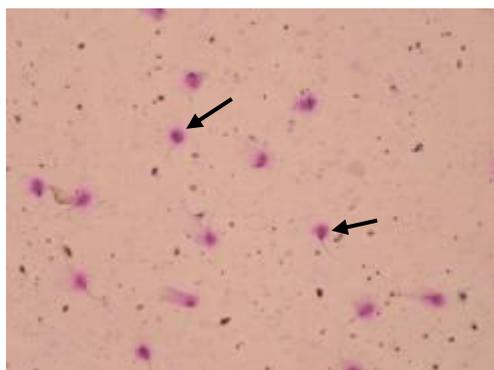


Figure 4. Halosperm <30% (x100) (original)

CONCLUSIONS

- Based on the results from patients diagnosed with normal spermia, we can say, after testing DNA fragmentation, that this test helps the specialist choose an effective AHR for the couples: it showed either that intrauterine insemination can be successful, or that IVF can be the solution because IUI would have been ineffective.
- Based on the results of the comparison of the sample of 9 patients diagnosed with oligospermia on which we tested ADN fragmentation with the sample that did not undergo the test, we can say that DNA fragmentation is useful and that it could be used as a routine method in AHR laboratories.
- Te goal of RUA is mainly to get women pregnant because it is extremely important psychologically, physically and financially. The new techniques can improve success percentage and reduce the time necessary to get women pregnant and avoid failure. The test meant to emphasise DNA fragmentation in human sperm is one of these techniques. This paper shows that the success percentage can be improved by adopting the AHR method depending on the result of the DNA fragmentation test.

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