

ANEUPLOIDY RATE IN SPERMATOZOA FROM MEN WITH ABNORMAL SEMEN PARAMETERS.

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INTRODUCTION

The frequency of constitutional chromosome aberrations in patients with oligoasthenoteratozoospermia (OAT syndrome) is higher respect those with normal semen parameters. Such chromosome anomalies are responsible for the production of aneuploid spermatozoa.

Recently, it was recognized that men with OAT syndrome with normal karyotype could have an increased aneuploidy rate, probably due to an altered intratesticular hormonal environment that could affect the chromosomal segregation during meiosis.

In spite of the FISH sperm study does not permit to analyse the complete chromosome complement, it is considered the best tool to estimate the production of aneuploid spermatozoa.

Objective:

The purpose of the present study was to estimate the sperm aneuploidy rate evaluating the aneuploidies of 13, 18, 21, X and Y chromosomes by sperm FISH from infertile patients with abnormal semen and fertile normozoospermic men.

Patients:

Fifty-three infertile men with abnormal semen parameters and 10 fertile men with normal semen parameters were studied. Both groups of patients had normal karyotypes.

According to the **total number of normal motile sperm (TNMS)** the patients were divided in three groups:

- Group A: less than 1×10^5 sperm/ml (n = 31)
- Group B: between 1×10^5 and 1×10^6 sperm/ml (n = 13)
- Group C: more than 1×10^6 sperm/ml. (n = 9)

Besides, the patients were divided regarding their sperm count, motility and morphology as follows:

Sperm Count

- A= Less than 5×10^6 sperm/ml (n = 16)
- B= Between 5×10^6 and 20×10^6 sperm/ml (n = 13)
- C= More than 20×10^6 sperm/ml (n = 24)

Sperm Motility

- A= Less than 20% Sperm Motility (n = 39)
- B= More than 20% Sperm Motility (n = 14)

Sperm Morphology

- A= Less than 15% of normal morphology (n = 24)
- B= More than 15% of normal morphology (n = 29)

Methods:

To perform the FISH study we used the in vitro decondensation sperm protocol with DTT. Co-Denaturation procedure was performed in the Hybrite instrument from Vysis™. The conditions were: **a)** Melting temperature 69°C during 8 minutes and **b)** Hybridization temperature 37°C during 24 hours.

Two mix of probes: **1)** CEP18+CEPX+CEPY and **2)** LSI13+LSI21 (Aneuvision kit from Vysis™) were used. After hybridization the slides were serial washed 10 min a 45°C in 50% formamide/2XSSC, 2XSSC and 2XSSC/0.01% tween followed by a wash in 2XSSC at rt during 5 min.

Fluorescent images were captured by a CCD camera.

At least 1000 sperm by mix of probes were analysed according to Martin & Radermarker recommendations including well define FISH signals and excluding the signal belonging to less decondensed sperm or with undefined borders.

The aneuploidy rate for the complete chromosome complement was calculated using the following math formula: $AR = ARXY \times [AR(13 \times 18 \times 21)^{1/3}]^{22}$ WHERE: AR= Aneuploidy rate.

RESULTS

Table 1: Aneuploidy sperm rate in OAT and Control.

Aneuploidy Rate					
Group	13	18	21	XY	Total
OAT	1.90 ± 2.59	0.64 ± 0.64	1.56 ± 2.40	1.70 ± 1.41	24.62 ± 18.09
Normal	0.58 ± 0.28	0.26 ± 0.11	0.55 ± 0.39	0.71 ± 0.29	10.09 ± 3.78

OAT ≠ Normal.

Table 2: Total normal motile sperm and Aneuploidy Rate.

Aneuploidy Rate					
Group	13	18	21	XY	Total
A	2.36 ± 2.60	0.79 ± 0.72	1.85 ± 1.82	2.10 ± 1.49	29.01 ± 17.93
B	1.28 ± 1.14	0.37 ± 0.38	0.91 ± 0.85	1.23 ± 1.19	17.65 ± 10.37
C	0.42 ± 0.27	0.46 ± 0.51	0.16 ± 0.10	0.91 ± 0.88	9.93 ± 8.55

A≠B≠C, except for 18 chromosome pair.

A= Less than 1×10^5 sperm/ml

B= Between 1×10^5 and 1×10^6 sperm/ml

C= More than 1×10^6 sperm/ml

Table 3: Sperm Count and Aneuploidy Rate.

Aneuploidy Rate					
Group	13	18	21	XY	Total
A	3.86 ± 4.51	0.73 ± 0.94	2.78 ± 3.97	2.10 ± 2.00	34.75 ± 26.08
B	1.34 ± 1.28	0.73 ± 0.58	1.19 ± 0.95	1.22 ± 1.02	20.70 ± 12.70
C	1.24 ± 1.12	0.62 ± 0.62	1.13 ± 1.33	1.63 ± 1.22	19.88 ± 13.33

A≠B and C, except for 18 chromosome pair

A= Less than 5×10^6 sperm/ml

B= Between 5×10^6 and 20×10^6 sperm/ml

C= More than 20×10^6 sperm/ml

Table 4: Sperm Motility and Aneuploidy Rate.

Aneuploidy Rate					
Group	13	18	21	XY	Total
A	2.10 ± 2.43	0.73 ± 0.68	1.74 ± 1.70	1.99 ± 1.47	26.98 ± 17.20
B	0.97 ± 1.09	0.34 ± 0.39	0.34 ± 0.26	0.81 ± 0.67	12.26 ± 8.15

A≠B

A= Less than 20% Sperm Motility

B= More than 20% Sperm Motility

Table 5: Sperm Morphology and Aneuploidy Rate.

Aneuploidy Rate					
Group	13	18	21	XY	Total
A	2.40 ± 2.72	0.65 ± 0.73	1.53 ± 1.61	2.01 ± 1.74	27.26 ± 17.73
B	1.39 ± 1.65	0.63 ± 0.58	1.30 ± 1.65	1.49 ± 1.10	20.64 ± 15.69

A=B except for 13 chromosome pair

A= Less than 15% of normal morphology

B= More than 15% of normal morphology

DISCUSSION

The cytogenetic study of human sperm is very difficult yet.

The complete analysis of the chromosomal set can be performed using the Hamster technique, which implies an in vitro fertilization procedure between denudated oocytes from hamster and human sperm. The outcome of this technique, mainly depends on the number of fertilized eggs and the quality of metaphases of the pronuclei. The few metaphases obtained and the high cost of this procedure have been the main reasons for sperm FISH study development.

Sperm FISH allows the study of a sufficient number of spermatozoa and comparatively is less expensive. The major inconvenient is that it doesn't permit the simultaneous analysis of the entire complement, although one

could use a mix of probes covering all chromosomes.

In the present study 5 chromosomes out of the 23 chromosomes were analysed. The chromosome elected were: 13, 18, 21, X and Y because they are the most common involved in human aneuploidies. Assuming that the 18 non studied chromosomes have a similar behavior that those studied, one could estimate the aneuploidy rate for all complement applying a mathematical formula.

Undoubtedly, the ICSI is the best option for severe male factor, but it is also true that an important proportion of those men rarely could get an own child, despite to try several ICSI cycles. An increase sperm aneuploidy rate could be a good explanation.

The average of aneuploidy rate in patients with OAT was $24,62 \pm 18,09\%$, ranging from 4 to 83%. In contrast, in fertile men was $10,09 \pm 3,78\%$, ranging from 4,2 to 14,3%.

As almost all patients had more than one altered semen parameter, we relate the aneuploidy rate with the TNMS. Table 2 clearly shows the increase of aneuploidy rate with the severity of the abnormal semen, finding the highest frequency in those with less than one hundred thousand of TNM sperm/ml.

Tables 3, 4 and 5 show the relationship between count, motility and morphology of the sperm with the percentage of aneuploidies. From these three parameters of the semen, the count and the motility were the most relevant.

The results obtained, allows us to conclude that the aneuploidy rate increases with the severity of abnormal semen parameters.

Thus, this kind of study should be done previous ICSI, mainly in severe male factor, to predict the outcome of the procedure and evaluate the possibility of PGD. In fact, 12 out of 37 patients achieved the pregnancy post ICSI. Eighty-three per cent had an aneuploidy rate less than 25%. The remaining 17% with more than 25% was benefited with the PGD.