Significance of the sperm DNA fragmentation index (DFI) for male fertility diagnostics

O. Vedrines, A. Mathwig, M. Stumm, Medicover Genetics, *MVZ Humangenetik Berlin-Lichtenberg, Berlin, Germany*

32. GfH-Jahrestagung
16.-18. März 2022, Würzburg

Background

A number of studies [1,2,3] around the world showed a decrease in sperm quality in men. Male infertility diagnostics is mainly based on analysis of clinical history, physical examination, hormone tests and sperm analysis.

Sperm analysis is an important part of **male infertility diagnostics**.

Sperm analysis involves analyzing 3 parameters:

- ✓ **The concentration of sperm cells (SCs)**
- ✓ **The percentage of motile SCs**
- ✓ **The percentage of normally formed sperm**

Sperm analysis does not consider **the integrity of the DNA molecule** in the sperm head.

We analyze:

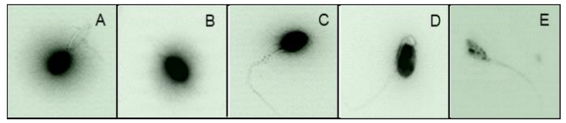
- How the age of the patient correlates with the DNA fragmentation in sperm.
- How the SCs DNA fragmentation correlates with the individual parameters of the spermogram.
- How the SCs DNA fragmentation correlates with spermogram as a whole.

Materials and Methods

Measure of the sperm DNA fragmentation index (DFI)

We compare the spermogram of 716 patients with their DNA Fragmentation Index (DFI). The DFI was assessed using the Halosperm® Test from Halotech, which is an indirect method to detect DNA strand breaks.

The DFI is the percentage of sperm cells in a semen sample that have fragmented DNA in their core.



Visual representation of different levels of DNA fragmentation inside sperm cells**. A, B:** Visible halo around the sperm cell head, underlining a low fragmentation. **C**: Small halo around the sperm cell head. **D**; No halo visible, **E**: Degraded sperm cell. **C, D, E** are typical representations of a high DNA representation of DNA fragmentation.

Distribution of patients in 3 groups

- **DFI ≤ 15%: P**regnancy with a fertile female partner can be achieved by **natural conception (NC)**. This group includes 399 patients.
- **DFI between 16 and 29%: I**ntra uterine insemination **(IUI)** is recommended. This group includes 220 patients
- **DFI ≥ 30%:** In Vitro Fertilization **(IVF)** or Intracytoplasmic Sperm Injection **(ISCI)** are recommended. This group includes includes 97 patients

Comparison of individual sperm analysis parameters in the 3 groups

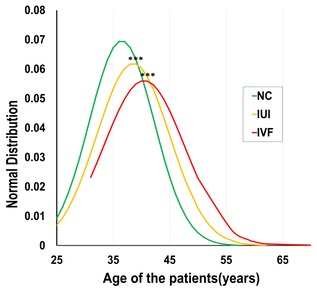
We analyzed the normal distribution of each parameter, performed t-tests to highlight the significance of our results, and calculated the correlation coefficient r² to determine, which sperm parameter is most impacted by the DFI.

Correlation between spermogram quality and DFI

Patients are divided into 4 groups according to the number (0, 1, 2, or 3) of abnormal spermogram parameters. The value of a parameter is considered abnormal when it is below the healthy range set by the WHO (2010)

Results

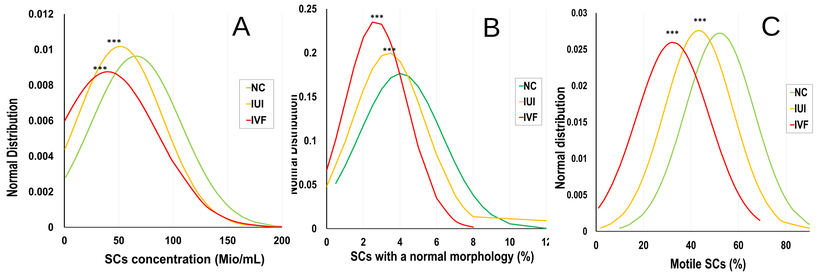
Correlation between age and DFI



Normal distribution of the age of the patients in the 3 DFI-categories of patients. The average ages are 36.5, 38.5 and 40.5 in the 3 DFI-based categories. **r²= 0.22.** ***p<0.002

There is a direct correlation between DFI and patient`s age: The DFI increases significantly with the age of the patients.

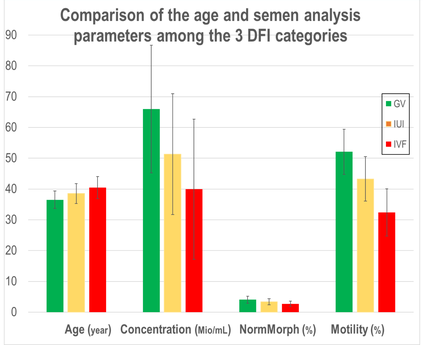
Correlation between individual sperm analysis parameters and DFI



Normal distribution of the 3 sperm analysis parameters in the 3 DFI-categories. **A**: Distribution of the SCs concentration. The average concentrations are 66.0, 51.3 and 39.9 in the 3 DFI-based categories. **r² =-0.21. B:** Distribution of the proportion of normally formed SCs. The average percentages are 4.04, 3.37 and 2.69 in the 3 DFI-based categories. **r² =-0.25. C:** Distribution of the proportion of motile SCs. The average percentages are 52.1, 43.3 and 32.4 in the 3 DFI-based categories. **r² =-0.46.** ***p<0.002

There is a correlation between DFI and the routinely tested sperm parameters: When the DFI increases, the concentration, the proportion of normally shaped and the motility of the SCs decreases.

Results summary



Graph highlighting the average in age, and sperm analysis parameters (concentration, percentage of normally formed sperm cells, and percentage of motile sperm cells) in the 3 different groups of patients. The scale on the y-axis is written in the bracket associated with each parameter.

Correlation between sperm analysis and DFI

Patients are distributed in 4 groups depending on the quality of their spermogram.

	3/3 parameters within normal		2/3 parameters within normal		1/3 parameters within normal		0/3 parameters within normal	
	Number of patients	(%)	Number of patients	(%)	Number of patients	(%)	Number of patients	(%)
NC: DFI <15	193	69.9	150	56.2	41	36.3	7	16.7
IUI: 15< DFI <30	71	25.7	89	33.3	39	34.5	15	35.7
IVF: DFI >30	12	4.3	28	10.5	33	29.2	20	47.6
Total	276	100	267	100	113	100	42	100

Among patients who present a healthy spermogram (all 3 parameters within normal ranges), we identify 12 patients (4.3%) whom DNA fragmentation inside their sperm cells are within critical range (higher than 30%)

patient ID number	DFI	Age (year)	Concentration (Mio/ml)	normo-morph (%)	Motility (%)
40555	30	73	40	7	46
HS36-13565	31	40	160	4	36.5
42641	32	**35**	73	4	41
36583	32	56	43	8	37
31365	32	46	85	4	36
35110	33	**37**	88	8	61
34392	34	43	77	5	41
33439	35	41	140	6	36
42763	36	**38**	28.5	4	45
38063	39	**34**	57	5	63
38445	40	41	78	6	56
40393	59	**35**	32	4	51
average	36.1	43.3	75.1	5.4	45.8

Out of those 12 patients, 5 of them are under 40 years old

Conclusions

- The DFI shows a direct correlation to the age of the patients.
- The DFI shows a correlation to all 3 standard spermogram parameters, alone and in combination.
- Interestingly, 4% of patients with a normal spermogram show a high DFI (>30%).

This highlights the fact that DNA fragmentation analysis can provide additional information that routine sperm analysis does not. Especially in patients with a normal spermogram and unexplained fertility problems, the DNA fragmentation index can serve as an **additional clinical biomarker**.

DFI analysis allows a **more detailed assessment of male fertility**. It can help to determine an individual and appropriate fertility treatment or explain previously unsuccessful therapeutic approaches

Acknowledgments & Citations

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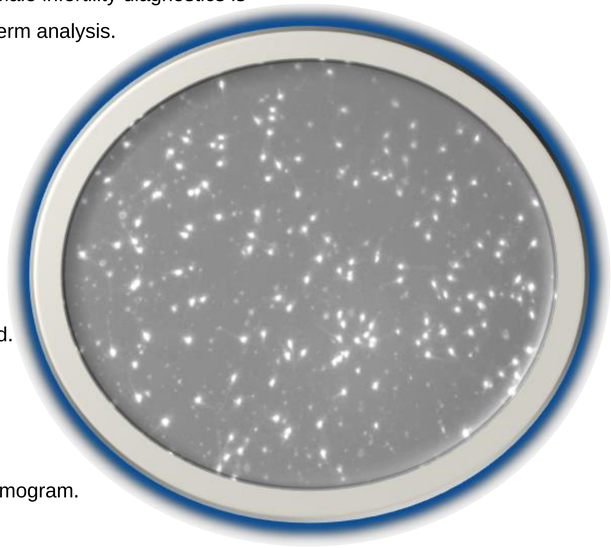

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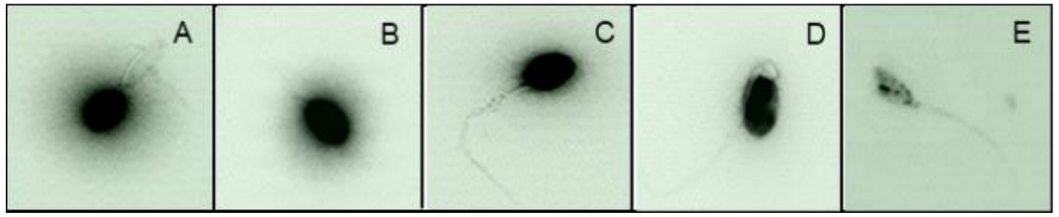

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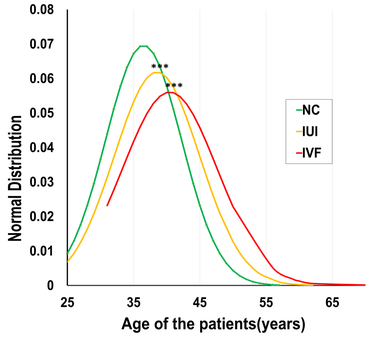

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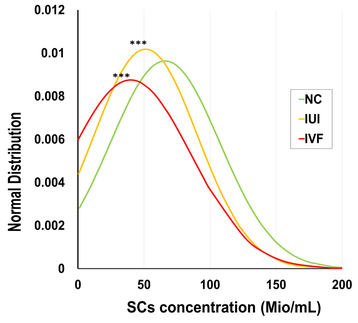

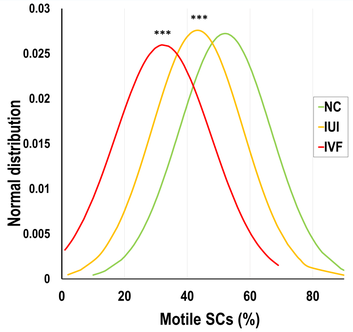

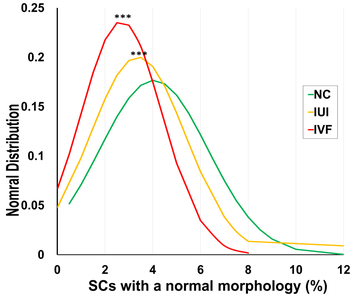

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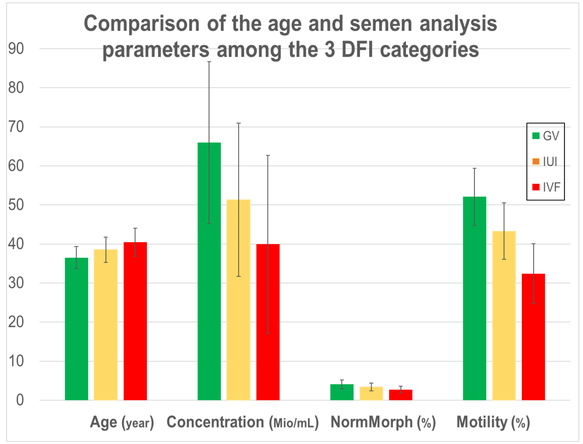

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Table showing the distribution of the patients in 4 groups depending on how many of the sperm parameters are within normal range.

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Average	**36**	**43**	**75**	**5**	**46**

Table representing 12 patients who had normal semen parameters but a high DNA fragmentation highlighted after performing the DNA dispersion assay.

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