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Sperm DNA Fragmentation Index in Abortion or *in Vitro* Fertilization Failure in Presence of Normal Semen Analysis

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Abstract: Role of male factor in recurrent abortion and *in vitro* fertilization failure has not been fully defined yet and there is much controversy about evaluating male patients with normal semen analysis. One of the factors that might help establish the male role is DNA fragmentation index. However, strong correlation between this factor and quality of semen, has caused many clinicians to believe that it does not help in abortion and implantation failure. We aim to assess this factor in our patients. In a prospective observational study, we assessed age, duration of infertility, undesired fertility related events (assisted reproductive techniques attempts and abortions), semen parameters and DNA fragmentation index in patients with multiple abortions or *in vitro* fertilization failures and analysed the results by statistical software SPSS version 24. DNA fragmentation index was remarkably correlated with age, duration of infertility and semen parameters. Among all groups in our study, patients with abnormal semen analysis had statistically significant higher level of DNA fragmentation. Ten percent of patients with normal or slightly abnormal semen analysis had abnormally high SDFI (sperm DNA fragmentation index). Checking DNA fragmentation index is recommended in all couples with fertilization problems even in the presence of normal semen analysis. It might be more reasonable to assess it in aged men, long duration of infertility or candidates with remarkable semen abnormality.

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Introduction

Infertility is a common problem for both the urologists and gynecologists. Although its treatment has been revolutionized by assisted reproductive techniques (ART), recurrent spontaneous abortion (RSA) and recurrent implantation failure (RIF) are still mysterious problems which are difficult to treat (ESHRE Guideline Group on RPL et al., 2018).

Role of male factor which contributes to 50% of infertility cases has not been established completely in these two conditions. Since sperm DNA fragmentation index (SDFI) was introduced, it has been used as an adjuvant assessment in RSA and RIF; however, we still have a long way to provide a comprehensive guideline for how and when to use it (Shaulov et al., 2020).

In this study we are presenting our findings regarding SDFI in patients with assisted reproductive techniques failure and abortion.

Material and Methods

In an observational prospective study, the information of couples who were referred to our infertility center because of RIF or RSA and had been assessed by history, physical examination, semen analysis, sperm DNA fragmentation index and karyotype, were collected. Inclusion criteria were normal karyotype, absence of azoospermia and at least three events of abortion and *in vitro* fertilization failure, or their combinations.

The patients were educated how to provide a standard semen sample.

Sperm count and motility were assessed by computer assisted semen analysis (CASA) (St. Petersburg 190000, Russia) and all specimens were controlled and corrected by an experienced andrology laboratory technician according to WHO (World Health Organization) 2010 criteria. Morphology report was based on a prepared separate sample after staining.

Simultaneously another part of semen was used for SDFI which was evaluated by Sperm Chromatin Dispersion (SCD) test using Sperm DNA Fragmentation Assay (SDFA) kit (Dianzystazma.cat 02 Tehran, Iran).

The information was collected by an anonymous information sheet from patients' files and was analysed by SPSS version 24. Inform consent was obtained from patients to use their information anonymously and the project was approved in Ethics Committee of Tehran University of Medical Sciences.

Results

During years 2017 to 2020, 172 couples were referred to our Andrology Clinic in order to be assessed for different combinations of infertility, ART failure and abortion.

Mean age of male candidate was 37.19 ± 6.5134 (minimum 23 and maximum 60). There was a positive relationship between age and SDFI (P-value = 0.035). Mean duration of infertility was 6.855 ± 5.0521 years with minimum of 12 months and

Table 1 – Number of patients and number of IVF failure or abortion

	1	2	3 and more	Total
IVF	13.37% (23)	16.86% (29)	22.09% (38)	(90)
Abortion	16.86% (29)	13.37% (23)	17.44% (30)	(82)
				100% (172)

IVF – *in vitro* fertilization

Table 2 – Mean of SDFI in patients related to cause and number of events

Number of events	1	2	3 or more	Average
IVF	25.95 ± 14.519	25.33 ± 13.924	28.79 ± 15.189	26.94
Abortion	22.41 ± 10.393	25.55 ± 16.772	22.68 ± 12.288	23.38

SDFI – sperm DNA fragmentation index; IVF – *in vitro* fertilization

maximum of 27 years. There was a strong positive correlation between duration of infertility and SDFI (P-value < 0.000).

Number and percentage of patients with *in vitro* fertilization (IVF) failure and abortion are demonstrated in Table 1.

There was not any meaningful relationship between SDFI and number of IVF failure or abortion. Mean of SDFI in separate groups are shown in Table 2.

Mean sperm count in this study was 21.32 ± 11.91 million/ml with maximum 56 and minimum 0.5 million per milliliter. 26.2% of patients had less than 15 million sperm/ml (oligospermia) and 73.8% of them had more than 15 million/ml (normozoospermia). There was a significant inverse relationship between SDFI and sperm count (P-value < 0.000).

Mean A + B motility in our patients was 28.13 ± 19.19% with minimum 0 and maximum 66%.

45.7% of patients had motility more than 32% (normal motility) and 55.3% of them had motility less than 32%. SDFI and motility had strong inverse relationship (P-value < 0.000).

Mean normal morphology was 3.25 ± 1.676 (minimum 0 and maximum 7%). 44.5% of patients had normal morphology less than 4% (teratospermia) and 55.5% of them had morphology more than 4%.

Morphology and SDFI had significant inverse relationship (P-value < 0.01).

Results of SDFI analysis in patients with normal or slightly abnormal semen analysis (47.7%) are shown in Table 3.

55 (32%) patients had normal semen analysis and 27 (15.7%) had abnormality only in one of three major factors of semen analysis (count, motility and morphology) which their SDFI levels are shown in Table 3.

Table 3 – SDFI in normal or slightly abnormal semen analysis in patients with multiple IVF failures or abortions

SDFI	<25	25–30	>30	Total
Completely normal semen analysis	63.41% (52)	1.2% (1)	2.4% (2)	67.0% (55)
Semen analysis with only one major factor abnormality	25.60% (21)	3.6% (3)	3.6% (3)	32.8% (27)
				100% (82)

SDFI – sperm DNA fragmentation index; IVF – *in vitro* fertilization

Discussion

RSA defined as 3 or more abortions and RIF with three or more IVF cycles failure have prevalence of 1% and 10% in fertility clinic respectively (ESHRE Guideline Group on RPL et al., 2018). Unfortunately, semen analysis parameters cannot predict them and defining the role of male partner in RIF and RSA becomes much sophisticated (Shaulov et al., 2020). Possible influencing factors such as paternal age, semen oxidative level and the genetic material of sperm (karyotype, chromatin structure and SDFI) have been reviewed in several studies without consistent result (Tan et al., 2019).

The significant relationship between age and duration of infertility with SDFI in our study indicates that patients of older age and longer duration of infertility should be screened for abnormal SDFI and may predict more profound problem. In several studies in addition to this finding the age of 40-years was suggested as a cut-off of SDFI checking (Humm and Sakkas, 2013; Gunes et al., 2016; Pino et al., 2020). According to our study even in younger males with long duration of these problems checking SDFI can be helpful.

In several studies, SDFI has been inversely related to spermiogram major parameters. Many physicians therefore believe that in these cases, SDFI does not provide clinicians with more information than semen analysis (Wiweko and Utami, 2017; Santi et al., 2018; McQueen et al., 2019). According to our survey, the decreased count, motility and sperm normal morphology are associated with higher SDFI. However, our findings (Table 3) indicate that failure to check the SDFI in the presence of normal or near-normal semen analysis results in the loss of 10% of patients with abnormal SDFI.

There are conflicting opinions in literature about the relationship of SDFI with RSA and RIF. In our study, the average amount of SDFI was very close to normal cut-off in overall and only in 3 and more IVF failures were reported to be slightly higher. The normal mean SDFI in our patients and similar studies can be explained by the multifactorial nature of fertility (Cho et al., 2017; Petersen et al., 2018; Yifu et al., 2020). The conflicting results regarding the association between SDFI and the

mentioned fertility problems can result from the simultaneous contribution of male, female conditions and also the laboratory factors in these cases are undeniable.

Deciding on the value of SDFI in RSA and RIF is difficult because SDFI has a proven relationship with the spermiogram, and both are inextricably linked to these two situations. Most likely, at least half of this lack of connection is related to the female factor and unknown causes.

Limitations

One of the factors that cause inconsistency in literature might be technique-related hence SDFI in one semen can be reported differently by various methods. We included all males without considering female factor. Taking into account the female factor adds to the accuracy of the study.

Another limitation was the time delay between last failure and semen evaluation, because most of couple were referred to us and were not our patients. Most importantly our study was retrospective with its own drawbacks.

Conclusion

Until a link is found between SDFI and fertility undesired events (RIF and RSA), SDFI measurement is recommended in these cases, even in the presence of a normal spermiogram, although an abnormal result might be found only in paternal aging, prolonged infertility, obvious spermiogram abnormalities and only in 10% of patients with normal semen analysis.

References

Cho, C. L., Agarwal, A., Majzoub, A., Esteves, S. C. (2017) Future direction in sperm DNA fragmentation testing. *Transl. Androl. Urol.* **6**, S525–S526 (Suppl. 4).

ESHRE Guideline Group on RPL; Bender Atik, R., Christiansen, O. B., Elson, J., Kolte, A. M., Lewis, S., Middeldorp, S., Nelen, W., Peramo, B., Quenby, S., Vermeulen, N., Goddijn, M. (2018) ESHRE guideline: Recurrent pregnancy loss. *Hum. Reprod. Open* **2018(2)**, hoy004.

Gunes, S., Hekim, G. N., Arslan, M. A., Asci, R. (2016) Effects of aging on the male reproductive system. *J. Assist. Reprod. Genet.* **33(4)**, 441–454.

Humm, K. C., Sakkas, D. (2013) Role of increased male age in IVF and egg donation: Is sperm DNA fragmentation responsible? *Fertil. Steril.* **99(1)**, 30–36.

McQueen, D. B., Zhang, J., Robins, J. C. (2019) Sperm DNA fragmentation and recurrent pregnancy loss: A systematic review and meta-analysis. *Fertil. Steril.* **112(1)**, 54–60.e3.

Petersen, C. G., Mauri, A. L., Vagnini, L. D., Renzi, A., Petersen, B., Mattila, M., Comar, V., Ricci, J., Dieamant, F., Oliveira, J. B. A., Baruffi, R. L. R., Franco, J. G. Jr. (2018) The effects of male age on sperm DNA damage: An evaluation of 2,178 semen samples. *JBRA Assist. Reprod.* **22(4)**, 323–330.

Pino, V., Sanz, A., Valdés, N., Crosby, J., Mackenna, A. (2020) The effects of aging on semen parameters and sperm DNA fragmentation. *JBRA Assist. Reprod.* **24(1)**, 82–86.

Santi, D., Spaggiari, G., Simoni, M. (2018) Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses. *Reprod. Biomed. Online* **37(3)**, 315–326.

Shaulov, T., Sierra, S., Sylvestre, C. (2020) Recurrent implantation failure in IVF: A Canadian Fertility and Andrology Society Clinical Practice Guideline. *Reprod. Biomed. Online* **41(5)**, 819–833.

Tan, J., Taskin, O., Albert, A., Bedaiwy, M. A. (2019) Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: A systematic review and meta-analysis. *Reprod. Biomed. Online* **38(6)**, 951–960.

Wiweko, B., Utami, P. (2017) Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. *Basic Clin. Androl.* **27**, 1.

Yifu, P., Lei, Y., Shaoming, L., Yujin, G., Xingwang, Z. (2020) Sperm DNA fragmentation index with unexplained recurrent spontaneous abortion: A systematic review and meta-analysis. *J. Gynecol. Obstet. Hum. Reprod.* **49**, 101740.