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Research Article

Influence of Male Personal and Demographic Characteristics on Semen Parameters and Intracytoplasmic Sperm Injection Outcome: Is Sperm DNA Integrity Testing Clinically Relevant?

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Abstract

The aim of the present study was to evaluate the impact of male characteristics on semen quality and to evaluate the impact of items of both parameters on ICSI outcome, with special focus on the clinical significance of sperm DNA integrity testing.

Patients and methods: This study included 100 non-azoospermic infertile men scheduled for ICSI. For each patient, full personal, demographic and medical data were collected and its relation to standard semen parameters, sperm DNA fragmentation (assessed by COMET assay) and ICSI outcomes were analyzed.

Results: Basic semen parameters were not correlated well with the studied male characteristics, except for the significant difference between hash users and non-users regarding seminal volume (p=0.019).

Among the studied male characteristics, the only parameters that correlated well with increased sperm mean DNA fragmentation index was increased husband's age and smoking status (p<0.000), however, pregnancy outcome after ICSI was not affected by the three parameters.

A significant statistical correlation was found between urban residency, chemical occupational exposure and increased female age with negative pregnancy outcome (p=0.018, 0.036 and 0.040 respectively), while there was no significant statistical correlation between pregnancy outcome neither with other male characteristics nor with standard semen parameters.

Conclusion: Paternal characteristics were not correlated well with conventional semen parameters. Although sperm DNA status was affected by smoking and aging, it does not influence ICSI outcome. Contrarily, other male characteristics (e.g. Urban residency and chemical occupational exposure) negatively correlated with pregnancy outcome without concomitant affection of sperm DNA status, which exclude DNA disruption as an explanation for the negative impact of paternal factors on ICSI.

Keywords: Male infertility; Personal history; Semen; Sperm DNA; COMET assay; ICSI

Introduction

Intracytoplasmic sperm injection (ICSI) represents a measurable therapeutic tool for infertile couple in which the influence of different factors on sperm - oocyte interaction and subsequent embryo development and pregnancy occurrence could be statistically analyzed.

Formerly, the success of ICSI (which needs only a single viable sperm) was thought to be independent of most of paternal factors, however, increasing evidences have suggested that ICSI outcomes may influenced by so far poorly characterized male-derived factors [1]. Although numerous male attributes were documented to affect male fertility, the impact of these factors on ICSI success is still poorly characterized.

Standard semen analysis, although providing data about some measurable sperm characteristics, is considered a poor indicator of fertility, as it does not measure more important functional aspects of sperm and sperm - oocyte interaction [2].

Assessment of sperm DNA damage was suggested by some researchers as a more accurate tool than conventional semen analysis for assessment of male fertility potential, although, the impact of sperm DNA damage on ICSI outcome in different studies was rather conflicting, mostly due to the heterogeneity of the related studies regarding assays used and couple's characteristics, inquiring the usefulness of routine application of DNA integrity testing before ART [3-11].

Accordingly, we sought to evaluate the impact of male characteristics on semen quality and intracytoplasmic sperm injection (ICSI) outcome, with special focus on the clinical relevance of sperm DNA integrity testing.

Methods

The current study was conducted at the Assisted Reproduction Unit, International Islamic Center for Population Studies and Research, Al-Azhar University; during the period between July 2017- and July 2018. The study had been approved by the Ethical Research Committee at the Dermatology, Venereology and Andrology Department, Faculty of Medicine for girls, Al-Azhar University. All patients in this study were informed about the study process and their written consents

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were taken prior to investigations, to use their clinical and laboratory information for analysis.

The study included 100 non-azoospermic male patients who were planning to undergo intracytoplasmic sperm injection (ICSI) for the 1st time.

Patients with azoospermia, hormonal dysfunction, apparent genetic abnormalities or secondary infertility were excluded from the study.

After gynaecological evaluation, only couples with female partners addressed as unexplained or male factor infertility were recruited to the study, while couples with female partners aged more than >35 years old or addressed as potentially poor responders (due to biochemical, clinical or sonographic factors that may negatively affect ICSI outcomes) were not included in the study. Also, during the ICSI process, female partners labelled as exaggerated or poor responders (Ovarion hyperstimulation or <2 metaphase II oocytes at ova pickup) were omitted to minimize the impact of female factor on the study results, and equal number of couples fulfilling the study criteria were engaged later on to substitute the dropped out members.

For each male patient full personal, demographic and medical data were collected. Personal data included male name, age, occupation (with focusing on occupations committed physical {heat, vibration or noise} or chemical {plastics, textiles, fertilizers, pharmaceuticals, pesticides, detergents, petrochemicals or painting products workers} exposures) and special habits (smoking and drugs abuse). Demographic data included urban or rural geographic location. Medical history included the history of systemic diseases and chronic medical treatments. Patients were subdivided according to age into two groups, > and <35 years old.

The ejaculate obtained from the male partner on the day of ova pick up, was divided into two unequal parts, the largest part was used for ICSI, while the remaining part used for semen analysis performed according to WHO criteria published in 2010 and assessment of DNA fragmentation [12]. In cases when the semen sample was inadequate, the whole sample was used for ICSI and the patient was asked to provide another semen sample for conventional semen assessment and DNA fragmentation assessment few days later .

Assessment of sperm DNA fragmentation by COMET assay

Principle: The COMET assay microscopically detects DNA damage at the level of a single cell with the aid of fluorescent DNA binding dye (Ethedium bromide (EtBr)). The dye measures sperm nuclear DNA fragmentation through binding to the double-stranded nucleic acids as an intercalating dye, so testing the DNA super coils for the possibility of breaks by causing negative DNA super coiling upon its addition, the loops expanded out from the nucleoid core would form a “comet”. Sperm DNA damage in this study was quantified by measuring sperm head fluorescent intensity, as previously described [13,14].

Technique for fluorescent microscopy study using the comet assay

1. Ethedium bromide preparation: The semen was stained with a diluted sample of ethidium bromide (EtBr to 0.5 µg/ml with Phosphate Buffer Solution (PBS).

2. Sperm staining with EtBr: All semen samples from participants were centrifuged at 1,500 rpm in the centrifugation chamber for 10 minutes. The supernatant of the seminal plasma was thrown away and sperms were washed by BPS and centrifuged again for 10 minutes.

The supernatant of BPS was thrown away followed by addition of ethidium bromide to sperms. A 5 minutes time was given to ensure proper DNA staining. One drop was placed on a slide then examined under the fluorescent microscope at X400 magnification. The slides were prepared on the same day with the fluorescent microscope using a 490-nm excitation filter and a 530-nm barrier filter. Increased sperm DNA fragmentation was expressed by decreased sperm head fluorescent intensity. At least 100 cells were counted so that estimate the percentage of spermatozoa with fragmented DNA (Figure 1 and figure 2). Comets were visually scored and given a percentage % score to evaluate sperm fluorescent fragmentation index. A single observer interpreted the fluorescent sperm head intensity to rule out inter-technician variability. The percentage of sperms with damaged DNA was expressed in terms of DNA fragmentation index (DFI):

<15% DFI = Excellent sperm DNA integrity

>15 to <25% DFI = Good sperm DNA integrity

>25 to <50% DFI = Poor sperm DNA integrity

>50% DFI = Very poor sperm DNA integrity

ICSI procedure

Ovarian stimulation was accomplished by a combination of gonadotropin-releasing hormone agonist and human menopausal gonadotropin. Ovulation was triggered by human chorionic gonadotropin, when at least three follicles measured 18 mm or more in diameter and serum oestradiol concentration were at least 1000 ng/L [15]. After oocytes retrieval, metaphase II oocytes were micro-injected with viable immobilized spermatozoa. After intracytoplasmic sperm injection, fertilization was defined as observation of two distinct pronuclei and two polar bodies 16-18 hours after micro-injection. Fertilized oocytes were observed for embryo development on days 2 and 3 after microinjection.

Cases that had positive fertilization and embryo development had embryo transfer on day 3. The primary outcome was clinical pregnancy rate; defined as sonographic detection of intrauterine viable pregnancy at 5th - 6th week after injection. Secondary outcomes included fertilization and embryo grade.

Statistical analysis

The data were collected and statistically analysed using Statistical Package for Social Science (SPSS) statistical program version 17.

Chi-square test (x2) used to study the correlation between two qualitative variables; Mann-Whitney test to compare between two groups not normally distributed having quantitative variables, and independent t-test to compare the means between two unrelated groups on the same continuous dependent variable. The significance level was set at 0.05.

Results

A total of 100 couples seeking fertility and undergoing ICSI, with husbands’ age ranging from 25 to 67 years and wives’ age ranging from 19 to 35 years. Pregnancy outcome of the studied patients showed positive clinical pregnancy in 24 patients (24%) and negative clinical pregnancy in 76 patients (76%).

There were no significant statistical relation between husband’s age, residence, smoking status, tramadol abuse or occupational exposures with basic semen parameters, while a significant difference between hash users and non-users was found regarding seminal volume (p=0.019) (Table 1).

	Male age		P-value	Residence		P-value	Smoking		P-value	Tramadol use		P-value	Hash use		P-value	Chemical exposure		P-value	Physical exposure		P-value
	<35 yrs No.=46	>35 yrs No.=54		Urban No.=54	Rural No. = 46		Non Smoker No.=46	Smoker No.=54		No No.=92	Yes No.=8		No No.=92	Yes No.=8		No No.= 68	Yes No.= 32		No No.=83	Yes No.=17	
Vol/ml Mean ± SD	2.26 ± 0.79	2.32 ± 0.92	*0.733	2.33 ± 0.95	2.25 ± 0.75	*0.615	2.21 ± 0.88	2.37 ± .85	*0.358	2.27 ± 0.88	2.54 ± 0.66	*0.406	2.23 ± 0.82	2.98 ± 1.10	***0.019**	2.26 ± 0.76	2.35 ± 1.06	*0.635	2.32 ± 0.89	2.18 ± 0.71	*0.544
Count- Mil/ml Median (Range)	45 (1–80)	45 (1–80)	*0.079	45 (1–80)	43 (1–65)	*0.208	45 (1–75)	40 (1–80)	*0.237	45 (1–80)	45 (3–65)	*0.904	45 (1–80)	30 (3–70)	*0.425	47.5 (1– 80)	32.5 (1– 80)	*0.112	45 (1–80)	45 (5–70)	*0.868
Total motility % Mean ± SD	40.71 ± 18.72	41.49 ± 18.55	*0.136	41.70 ± 17.96	40.59 ± 19.92	*0.769	42.46 ± 9.83	40.11 ± 17.99	*0.537	40.86 ± 19.00	45.00 ± 16.90	*0.553	41.78 ± 18.78	34.38 ± 18.79	*0.287	41.71 ± 18.24	40.09 ± 20.17	*0.691	39.81 ± 18.87	47.94 ± 17.42	*0.104
Abnormal morphology % Mean ± SD	90.61 ± 8.71	87.41 ± 12.24	*0.141	88.85 ± 9.61	88.91 ± 12.22	*0.978	87.11 ± 3.06	90.39 ± 8.31	*0.132	88.42 ± 11.08	94.13 ± 5.36	*0.154	88.68 ± 10.57	91.13 ± 14.17	*0.544	88.18 ± 11.66	90.38 ± 8.77	*0.346	89.20 ± 11.11	87.29 ± 9.46	*0.510
No. of fertilized Ova Mean ± SD	2.93 ± 1.44	2.65 ± 1.14	*0.268	2.76 ± 1.18	2.80 ± 1.41	*0.862	2.78 ± 1.23	2.78 ± 1.34	*0.985	2.82 ± 1.29	2.38 ± 1.19	*0.355	2.79 ± 1.32	2.63 ± 2.08	*0.724	2.87 ± 1.40	2.59 ± 0.98	*0.322	2.80 ± 1.30	2.71 ± 1.21	*0.795

Table 1: Correlation between male characteristics and basic semen parameters.

*:Independent t-test; •:Mann-Whitney test.

A highly significant statistical relation was found between increase husband's age (>35 years) and smoking status with the increase in mean DNA fragmentation index (p<0.000) and increase in percentage of very poor DNA fragmentation index (p<0.001), while there was no significant statistical relation between sperm DNA fragmentation index with other male characteristics (Table 2).

There was no significant statistical correlation between degree of DNA fragmentation with ICSI outcomes, including number of fertilized oocytes, embryo grades (a, b and c) or pregnancy occurrence (p= 0.587, 0.201, 0.358, 0.157 and 0.414 respectively) (Table 3).

A significant statistical correlation was found between increased female age, urban residency and chemical occupational exposure with negative pregnancy outcome (p=0.040; 0.036 and 0.018 respectively), while there was no significant statistical difference between patients with positive and negative pregnancy outcome regarding other patients' characteristics or semen parameters including mean DNA fragmentation index (Table 4).

Discussion

Male factor-related infertility is a rising phenomenon among infertile couples. There is an ongoing effort to understand the factors that affect sperm quality and subsequently affect ART outcome. Apparently, the alterations in sperm quality can be related to habits, such as lifestyle, obesity, smoking, alcohol and drug intake, which were suggested to negatively affect semen quality. Environmental hazards as well as occupational exposure have been declared as important factors acting to decrease sperm quality. Moreover, physiologic factors as race, ethnicity, geographic location or even seasonal variations can be related to sperm analysis [16].

Although intracytoplasmic sperm injection (ICSI) was designed to overcome all forms of male infertility, repeated failure may be due to abnormal semen quality, mostly unrecognizable by conventional semen analysis. Standard semen analysis, although providing data about sperm characteristics, is considered an inaccurate measurement of fertility, as it does not measure functional aspects of sperm transport in the female genital tract or sperm - oocyte interaction [17]. Evaluation of sperm DNA status was recommended by some investigators as a more precise tool for assessment of male fertility, however, the impact of sperm DNA damage on ICSI outcome among different studies was inconsistent [9,11].

Our study aimed at evaluating the impact of male age, occupation,

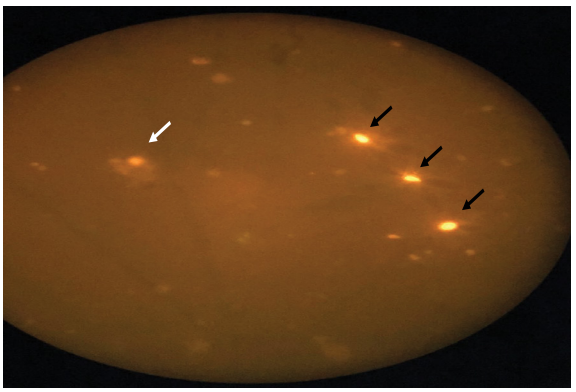

Figure 1: Black arrow showing normal sperm chromatin and white arrow showing abnormal sperm using Ethedium Bromide stain, under fluorescent microscope.

		DNA fragmentation index Mean ± SD Range	P-value	DNA fragmentation index				P-value
				Excellent	Good	Poor	V.poor	
Male age	**< 35 yrs No.=46**	49.89 ± 21.72 10–100	***0.000***	3 (6.5%)	10 (21.7%)	14 (30.4%)	19 (41.3%)	X^2***0.001***
	> 35 yrs No.=54	67.31 ± 17.26 10–90		1 (1.9%)	1 (1.9%)	11 (20.4%)	41 (75.9%)	
Residence	**Urban No. = 54**	56.57 ± 23.19 10–90	*0.165	3 (5.6%)	9 (16.7%)	10 (18.5%)	32 (59.3%)	$X^2$0.106
	Rural No. = 46	62.50 ± 18.37 10–100		1 (2.2%)	2 (4.3%)	15 (32.6%)	28 (60.9%)	
Smoking	**Non Smoker No.=46**	50.54 ± 20.36 10–100	***0.000***	2 (4.3%)	9 (19.6%)	17 (37.0%)	18 (39.1%)	X^2***0.001***
	Smoker No.=54	66.76 ± 19.11 10– 90		2 (3.7%)	2 (3.7%)	8 (14.8%)	42 (77.8%)	
Tramadol use	**No No.=92**	58.37 ± 21.55 10–100	*0.138	4 (4.3%)	11 (12.0%)	23 (25.0%)	54 (58.7%)	X^2 0.653
	Yes No.=8	70.00 ± 13.63 50–90		0 (0.0%)	0 (0.0%)	2 (25.0%)	6 (75.0%)	
Hash use	**No No.=92**	58.21 ± 21.36 10–100	*0.080	4 (4.3%)	11 (12.0%)	24 (26.1%)	53 (57.6%)	$X^2$0.400
	Yes No.=8	71.88 ± 15.34 50 – 90		0 (0.0%)	0 (0.0%)	1 (12.5%)	7 (87.5%)	
Chemical exposure	**No No.= 68**	58.01 ± 21.53 10 – 100	*0.380	3 (4.4%)	8 (11.8%)	20 (29.4%)	37 (54.4%)	$X^2$0.397
	Yes No.= 32	62.03 ± 20.59 10 – 90		1 (3.1%)	3 (9.4%)	5 (15.6%)	23 (71.9%)	
Physical exposure	**No No.=83**	60.60 ± 21.76 10 – 100	*0.176	4 (4.8%)	8 (9.6%)	18 (21.7%)	53 (63.9%)	$X^2$0.172
	Yes No.=17	52.94 ± 17.51 25– 80		0 (0.0%)	3 (17.6%)	7 (41.2%)	7 (41.2%)	

*:Independent t-test; X^2: Chi-square test.

Table 2: Correlation between patients characteristics and sperm DNA fragmentation.

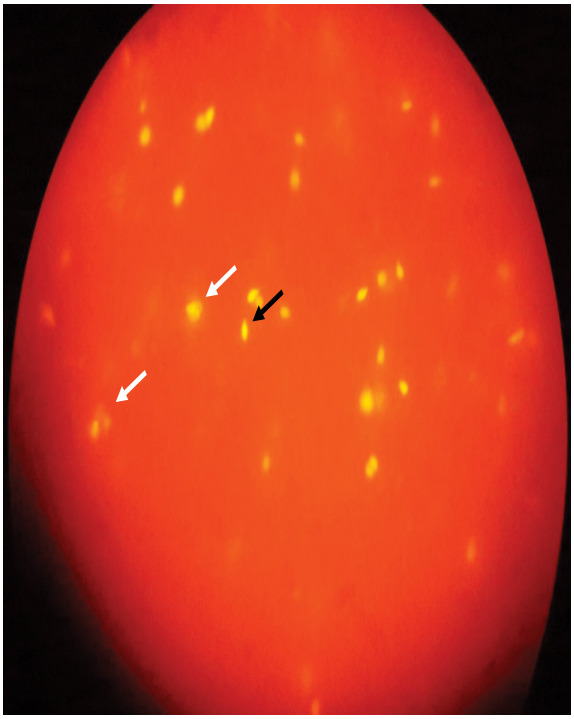

Figure 2: Black arrow showing normal sperm chromatin and white arrow showing abnormal sperm using Ethedium Bromide stain, under fluorescent microscope.

residence, medical history and special habits on semen quality (including basic semen parameters and sperm DNA fragmentation), and consequently on pregnancy outcome among couples undergoing ICSI. The study included 100 infertile males undergoing ICSI. For each patient, data about age, geographic location, special habits, occupation and medical history were collected. Semen samples were collected and analyzed according to WHO criteria published in 2010. Then an assessment of the DNA fragmentation index was done using Ethedium bromide dye under fluorescent microscope (COMET assay).

In our study, basic semen parameters, were not correlated well with the considered paternal features. Among the studied male characteristics, the increased husband's age and smoking, status were the only parameters that correlated well with the increased sperm mean DNA fragmentation index, although, this was not reflected on the pregnancy outcome, which on the other hand was significantly associated with other studied characteristics, namely the urban residency, chemical occupational exposure and increased female age, which committed this negative impact on pregnancy outcome without concomitant affection of the sperm DNA status, while there was no significant statistical correlation between pregnancy outcome neither with other male characteristics nor with standard semen parameters.

Some previous studies had shown an inverse correlation between male age and conventional semen parameters, while others, in agreement with our results, failed to observe connections between any change in basic semen parameters with advanced paternal age [18,19]. The dissimilarity in results between studies may be due to heterogeneity of these studies regarding study populations and sperm evaluation methodologies [20].

Although, our study has shown no significant statistical correlation between husband's age and conventional semen parameters, husband's age was significantly statistically correlated with an increase in poor DNA fragmentation index. In agreement with our results, other

DNA fragmentation index		Excellent	Good	Poor	V. poor	P-value
		No.= 4	No.= 11	No.= 25	No.= 60	
Number of Fertilized ova	Mean ± SD	2.25 ± 0.96	3.18 ± 1.47	2.84 ± 1.46	2.72 ± 1.19	*0.587
Grade (a) embryos	Mean ± SD	2.33 ± 0.58	2.43 ± 1.40	2.09 ± 1.51	1.70 ± 0.80	*0.201
Grade (b) embryos	Mean ± SD	1 ± 0	1.38 ± 0.52	1.25 ± 0.45	1.58 ± 0.77	*0.358
Grade (c) embryos	Mean ± SD	–	1.50 ± 0.58	1.00 ± 0.00	1.18 ± 0.41	*0.157
Pregnancy outcome	No pregnancy	2 (50.0%)	10 (90.9%)	19 (76.0%)	45 (75.0%)	X^2 0.414
	Positive pregnancy	2 (50.0%)	1 (9.1%)	6 (24.0%)	15 (25.0%)	

*: Independent t-test; X^2: Chi-square test.

Table 3: Relation between degree of DNA fragmentation with ICSI outcomes.

Patients' characteristics		No pregnancy	Positive pregnancy	X^2/t*/Z•
		No.=76	No.=24	P-value
Wife age	Mean ± SD	30.38 ± 4.10	28.38 ± 4.21	***0.040***
	Range	19–35	20–35	
Husband age	Mean ± SD	37.28 ± 8.61	34.96 ± 8.36	*0.250
	Range	25–60	27–67	
Residence	Urban	46 (60.53%)	8 (33.3%)	X^2***0.036***
	Rural	30 (39.47%)	16 (66.7%)	
Smoker	Non smoker	34 (44.7%)	12 (50.0%)	$X^2$0.783
	Mild	8 (10.5%)	1 (4.2%)	
	Moderate	8 (10.5%)	2 (8.3%)	
	Severe	26 (34.2%)	9 (37.5%)	
Special habits	Tramadol	7 (9.2%)	1 (4.2%)	$X^2$0.427
	Hash	8 (10.5%)	0 (0.0%)	$X^2$0.097
Computer user	No	67 (88.2%)	20 (83.3%)	$X^2$0.540
	Yes	9 (11.8%)	4 (16.7%)	
Hypertension	No	75 (98.7%)	24 (100.0%)	$X^2$0.572
	Yes	1 (1.3%)	0 (0.0%)	
Diabetes mellitus	No	73 (96.1%)	23 (95.8%)	$X^2$0.962
	Yes	3 (3.9%)	1 (4.2%)	
Hepatitis B virus	No	74 (97.4%)	24 (100.0%)	$X^2$0.422
	Yes	2 (2.6%)	0 (0.0%)	
Hepatitis C virus	No	75 (98.7%)	23 (95.8%)	$X^2$0.384
	Yes	1 (1.3%)	1 (4.2%)	
Medical treatment	No	72 (94.7%)	23 (95.8%)	$X^2$0.830
	Yes	4 (5.3%)	1 (4.2%)	
Chemical exposure	No Yes	47 (61.8%) 29 (38.2%)	21(87.5%) 3 (12.5%)	X^2***0.018***
Physical exposure	No Yes	64 (84.2%) 12 (15.8%)	19 (79.2%) 5 (20.8%)	$X^2$0.566
Psychological exposure	No Yes	52 (68.4%) 24 (31.6%)	15 (62.5%) 9 (37.5%)	$X^2$0.591
Semen parameters				
Vol (ml)	Mean ± SD	2.30 ± 0.93	2.27 ± 0.64	*0.886
	Range	0.5–5	1–3.5	
Count (million/ml)	Median (IQR)	45 (15.0-55.0)	47.5 (25.0–57.5)	•0.535
	Range	1–80	5–70	
Total motility %	Mean ± SD	40.89 ± 19.12	42.13 ± 18.07	*0.781
	Range	0–70	1–70	
Abnormal morphology %	Mean ± SD	89.33 ± 9.43	87.46 ± 14.56	*0.463
	Range	60–100	30–100	
DNA fragmentation index	Mean ± SD	59.54 ± 21.51	58.54 ± 20.67	*0.842
	Range	10–100	10–90	

*:Independent t-test; •:Mann-Whitney test; X^2: Chi-square test.

Table 4: Correlation between patients' characteristics and pregnancy outcome.

investigators confirmed that the percentage of highly damaged DNA sperm in male age group 36-57 years was significantly higher compared to the age group 20-35 years, concluding a positive correlation between increasing male age and DNA damage which may be attributed to

increase in ROS production or ineffective antioxidant scavenging system in elderly men [21-23].

Also, our study found no significant statistical correlation between smoking with standard semen parameters. However, very poor DNA fragmentation index was highly statistically correlated with smoking. In agreement with our study, De Jong et al. found no statistically significant correlation between cigarette smoking with conventional semen parameters, as the proposed effect of smoking on semen parameters could be found between smoker and non smoker infertile men [24]. Cui et al also found that tobacco smoking causes DNA damage and subsequently elevate DNA fragmentation rates [25].

In the current study, in accordance with others, both parameters (male ageing and smoking) seems to have no impact on pregnancy outcome after ICSI, despite their deleterious effect on sperm DNA status, which was suggested by other investigators to be the incriminated link between paternal factors and their negative impact on ICSI outcome [24,26]. However, according to our results, sperm DNA integrity appears to have no negative impact on ICSI results, which is in accordance with other studies, which demonstrated that the pregnancy rate is not correlated with DFI, and pregnancies occurred even with neat semen samples characterized by a high DFI >30% [27-29].

This may be explained by the fact that semen preparation using swim-up or density gradient methods during the ICSI procedure may exclude DNA fragmented spermatozoa from the selection process and increase the proportion of spermatozoa with intact DNA, leading to a more probability to use a normal spermatozoa in the injection process [29]. Also, despite the wide range age of male partners participating in the study, all female partners were under 35 years, and it was previously suggested that oocytes of young women are capable of repairing limited endogenous and exogenous DNA damage of paternal origin, which may represent the second-line defence against the negative impact of sperm DNA damage on embryo development and pregnancy outcome after ICSI. However, this feature may be lost gradually with female aging, which support the results of the present study that husband's age, even with increased sperm DNA fragmentation, has no significant effect on pregnancy outcome, while wife's age has [30-33]. Moreover, the selection process of the best quality embryos for transfer during ICSI may diminish the potential risk of sperm DNA damage on ICSI outcome. Consequently lower sperm DNA integrity, according to meta-analysis of ART studies, is associated with lower natural (but not ICSI) pregnancy rate [3,8,34-36].

In the same way the absence of correlation between sperm DNA damage and other ICSI outcomes as fertilization and embryo quality may be explained. Moreover, it was suggested that both fertilization and early embryonic growth is not affected by sperm DNA status [29,37-39], as the paternal genome is activated only after the second embryonic division [6,40].

For the effect of the location of residency on semen parameters, we found no significant statistical difference between urban and rural residents as regards basic semen parameters or DNA fragmentation index. Although, a significant statistical correlation between positive pregnancy outcome with rural residency was observed.

In a study by Zhou et al. on a study population from China, they observed better male semen quality in the rural area than in the urban area [41]. On the other hand, a study by swan et al. suggested that semen quality may be reduced in rural areas relative to urban areas in the US [42]. To resolve this discrepancy, some investigators suggested that the influence of geographic location on reproductive function depends on the genetic background of the study population that may predispose to, or guard against the insult [43].

Also, in agreement with our results, Frutos made a study on patients undergoing ICSI with normal semen parameters, and found a significant correlation between air pollution exposure in urban areas and miscarriage. The authors suggested that the direct transfer of pollutants through the placenta could lead to hypoxic damage and even immune-mediated injury at a critical moment of the embryo, which could potentially result in a negative pregnancy outcome [44]. However, this explanation refers to a female rather than a male affection.

In our study, regarding special habits, tramadol use had no significant statistical correlation with semen parameters, DNA fragmentation index or pregnancy outcome. These findings supported by a study conducted by Azari and coworkers, who found no significant correlation between tramadol use and semen parameters. Moreover, they reported that the mild and statistically insignificant effects of tramadol on semen parameters in their study were dose dependent and reversible, as all semen parameters tended to return to normal values after 6 weeks cessation of the drug [45]. however, it should be stressed that, most of the addict patients deny or not mentioning drug abuse as a part of their history, so, in the current study, it was difficult to make a firm conclusion about effect of tramadol on reproductive function.

For the effect of hash use, there was a significant statistical correlation between higher mean semen volume with hash use, while there was no significant statistical correlation between hash use and other semen parameter, DNA fragmentation or pregnancy outcome.

In a study by Plessis et al. on 1700 patients they reported, in contrast to our results that hash exposure is a risk factor for poor sperm morphology, but hash no effect on semen volume [46]. The heterogeneity in results between studies regarding morphology affection may be due to heterogeneity of these studies regarding the dosage of hash administration, study population and sperm evaluation methodologies.

No significant statistical correlation was found between chronic medical illnesses (hypertension, diabetes and chronic viral hepatitis), and semen parameters, DNA fragmentation index and pregnancy outcome in our study. In agreement with our results, Agbaje et al. did not find any significant difference in semen parameters and pregnancy outcome in patients with DM compared to non-diabetic controls. They concluded that DM is not a direct cause for infertility, but infertility may be an indirect consequence of its complications as poor metabolic control, associated neuropathy or erectile dysfunction [47].

Although some studies discuss the role of DM and hypertension on male fertility, but no sufficient studies about the effect of their medications on male fertility [48]. We could not find a significant statistical correlation between chronic drug intake with semen parameters, DNA fragmentation or pregnancy outcome, although, this may be attributed to the small number of patients on chronic medical therapy in our study population.

For the effect of different occupational exposures, our results showed no significant statistical correlation between occupational (chemical and physical) exposures with basic semen parameter, however chemical occupational exposure was significantly statistically correlated with decreased pregnancy outcome, despite the absence of significant negative impact of chemical exposure on DNA fragmentation index. This negative impact of chemical occupational exposure on pregnancy outcome is supported by our results regarding the negative effect of urban residency (which supposed to have more pronounced chemical exposure) on pregnancy outcome. On the other hand, there was no statistical significant correlation between physical exposure and pregnancy outcome or DNA fragmentation index.

In contrast with our study, Melgarejo et al. found that exposure to chemical compounds may be associated with decreased sperm counts and motility [49]. Also, Aguilar-Garduño et al. reported an association between chemical exposure and abnormal semen parameters [50]. Moreover, a study by Dhanushka & Peiris, in contrast to our results, suggested that chemical occupational exposure may lead to increase in DNA fragmentation index [51]. The discrepancy between the results of these studies and our results regarding the effect of chemical occupational exposure on semen parameters and sperm DNA may be attributed to the huge variations in types and magnitude of exposure to chemical compounds for each individual, with consequently unequal effects on reproductive function.

An explanation for the fact that, pregnancy outcome was the only affected parameter by chemical occupational exposure among other ICSI outcomes, comes from a study by Guimarães who found that; chemical compounds passes the placenta leading to spontaneous abortion and miscarriage [52], which may explain the decreased pregnancy outcome with chemical exposure, even though semen parameters, DNA fragmentation index, fertilization and embryo quality were normal in our study. Considering that both partners in the same couple most likely share a common chemical environmental exposures, this may indicate a maternal post-implantation rather than a paternal pre-implantation effect of chemical exposures or may indicate a paternal yet-undefined delayed effect of chemical exposure on pregnancy outcome.

In accordance with our results, Eisenberg et al. found that physical occupational exposure (vibration, noise, heat or prolonged sitting) was not correlated with semen quality, as these factors are not stable all the time, and also due to the testicular thermo-regulation system which is certainly able to maintain the normal scrotal hypothermy [53].

In the current study, we did not observe any significant statistical correlation between basic semen parameters and pregnancy outcome after ICSI.

In agreement of our results, a study by Shabtaie et al. has reported no correlation between sperm morphology and pregnancy outcome [54]. In contrast, another study by Franken had shown a significant correlation between morphology and positive pregnancy [55]. Also, sperm morphology was found to be a prognostic factor for ICSI outcome by other investigators [56].

The main reason for the discrepancy between these studies is probably related to the heterogeneity of the studies with regard to the techniques and cut off values used in morphology assessment. Also, abnormal morphology by definition refers to a numerous dissimilar types with mostly unequal consequences rather than a single entity and some types of sperm abnormalities (especially related to sperm head) may represent a risk factor for assisted reproduction than other abnormalities [57].

Conclusion

In our study, ICSI outcome was not related to basic semen parameters. Also ICSI seems to be able to overcome the negative impact of some paternal factors on sperm DNA, which may be explained by the effectiveness of sperm and embryo selection methods used in ICSI or indicate a possible fixation ability of the oocytes to limited sperm DNA injuries. Other paternal factors seem to impair pregnancy outcome after ICSI without a concomitant impairment of sperm DNA status, which point towards an accused mechanisms other than genetic material injury. According to our results, the routine sperm DNA integrity testing before ICSI cannot be recommended.

Limitations

Despite our efforts to minimize the maternal effect on the study results, maternal factor cannot be completely excluded as a contributing factor in the effect of male residence and environmental occupational exposure on ICSI outcome, considering that both partners most likely share a common residence and environmental exposures, which may lead to both maternal and paternal impact on ICSI outcome. More well designed studies are needed to precisely detect the paternal factors that may influence ART outcomes.

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