

THE INTERACTION OF FEMALE AGE AND ACTIVE MALE SMOKING HAS NEGATIVE INFLUENCE ON SUCCESS RATES OF THE *IN VITRO* FERTILIZATION TREATMENTS

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ABSTRACT

This study monitors the effect of male smoking and age of the woman on the success of the intracytoplasmic sperm injection (ICSI) fertilization process as part of the assisted reproduction technique (ART). A total of 703 couples in the *in vitro* fertilization (IVF) program were included. Binary logistic regression analysis was used to study the effect of male smoking on clinical pregnancy rate and live birth rate. The results from the study showed that interaction of male smoking and women's age (>35 years) have significant negative impact on ongoing pregnancy rate and live birth rate.

Keywords: Assisted reproduction treatment (ART); Infertility; Intracytoplasmic sperm injection (ICSI); *In vitro* fertilization (IVF); Male smoking.

INTRODUCTION

Many variables have influence on the final success in the *in vitro* fertilization (IVF) process. In recent years, attention to human reproduction is how lifestyle factors such as cigarette smoking have impact on IVF outcome [1].

It has been found that cigarette smoke contains cotinine, cadmium, metabolites of nicotine that are toxic, mutagenic chemicals and carcinogens. These chemicals cross the blood-testis barrier the proof of this is the concentration level of these chemicals in seminal plasma that are proportional to those in the serum, this provides genotoxic environment for germ cells [2].

This genotoxic environment causes oxidative DNA damage in spermatozoa due to its high content of oxidants

[3]. Oxidative stress (OS) from smoking is causing genetic and epigenetic changes that may correlate directly with reduced sperm parameters and reduced sperm function [4]. Spermatozoa are unable to restore the damage induced by OS because they lack the necessary cytoplasmic-enzyme repair systems [5]. Sperm DNA is more sensitive to OS and produces base-free sites, deletions, frameshift mutations, DNA cross-links and chromosomal rearrangements that contribute to apoptosis, poor fertilization rate, high frequency of miscarriage and morbidity in offspring in active male smoking, in addition to active and passive female smoking [4,6,7]. Some lifestyle factors in female patients such as smoking may cause irregularity in menstrual cycles [8], as well as decreasing ovarian reserve [1,9].

The aim of this study was to explore the influence of male smoking and female age on the success of IVF procedures. The final IVF result (clinical pregnancy, ongoing pregnancy rate and live birth rate) depends on several variables; therefore, the analysis in this study included exclusion criteria. Basic hypothesis of this study was to determine if the male smoking in interaction with the age of female patients has a negative impact on the outcome of IVF, as seen by decreased clinical pregnancy rate and live birth rate.

MATERIALS AND METHODS

This prospective study was during the period from January 2012 to December 2013, total number of 703 cycles were included, the patients completed anonymous questionnaires concern smoking status. The study was approved by the Ethics Committees of the hospital. One of the factors with a negative significant impact on the IVF results was the age of the female patients leading to diminished ovarian reserve. According to the European Society of Human Reproduction and Embryology (ESHRE) defi-

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nitition for poor ovarian response, we used more stringent exclusion criteria such as ovarian reserve tests and patient age [10]. Therefore, patients above 39 years of age were excluded from the analysis, as well as patients with basal follicle-stimulating hormone (bFSH) levels greater than 10 mIU/mL, Anti-Müllerian hormone (AMH) less than 1 ng/mL, and with less than five pre-antral follicles on vaginal ultrasound. Female patients were divided into two groups according to their age: younger or equal of 35 years of age and 36 years to 39 years old. The border of 35 years old is a significant threshold with regard to the IVF success rate, because poor ovarian reserve generally accompanied by the age of women are decreasing the quantity and quality of oocytes as well as embryos, which lead to low implantation and high miscarriage rates [11-14].

The male exclusion criteria were azoospermia, severe oligozoospermia (<1 million sperm cells per milliliter of ejaculate), sperm donation, chromosome alteration (Klinefelter syndrome), factors that may increase the temperature in the scrotum (fever, cryptorchidism, varicocele), exposure to environmental toxicants (dyes, chemicals and drugs affecting patient's sperm analysis); leukocytosis indicating a genitourinary infections (anamnesis of a positive sperm culture) before the start of controlled ovarian stimulation (COS), and finally genitourinary operations or hernia. Patients treated with chemotherapy or having endocrine diseases such as diabetes mellitus were also excluded from the study.

A long agonist stimulation protocol was used in all patients. Down-regulation was made with gonadotropin releasing agonist (GnRh-a), [Buserelin or Suprefact®) and ovarian stimulation with recombinant gonadotropins (rFSH)] (follitropin- β or Puregon®), or urinary gonadotropins (uHMG) (Menopur®) (Ferring Pharmaceuticals, Saint-Preix, Switzerland). Depending on the patient's age and pre-antral follicles number, a starting dose of 150 to 600 IE was used. The ampule of 10,000 IU hCG (human chorionic gonadotropin) (Pregnyl®; MSD Nederland, Oss, The Netherlands) was administrated as a final oocytes maturation trigger when at least two follicles were the diameter of >18 mm detected on vaginal ultrasound.

Aspiration of the follicles was made with single lumen aspiration needle under control of vaginal ultrasound approximately 36 hours after the triggering. All retrieved oocytes after preparation were cultured and after 2 hours from collection denudation was made [15]. All mature oocytes (meta-phase II) were fertilized with intracytoplasmic sperm injection (ICSI). A density gradient method was used for semen sample purification density [16]. Embryos were classified according to the scoring system of Hardarson *et al.* [17]. Embryo transfer was made on the third or fifth day according to the scoring system of quantity and

quality of the embryos [18], (transcervical transfer under the ultrasound control). Progesterone was given to all patient as a luteal support. Pregnancy tests were completed the 14th day after embryo transfer by measuring the serum β -hCG. Two weeks after the positive pregnancy test, vaginal ultrasound was used to confirm clinical pregnancy.

Statistical Analyses. Statistical analysis was performed using the Statistical Package for the Social Sciences (version 13.0) (SPSS Inc., Chicago, IL, USA). Categorical variables were analyzed with the χ^2 test and the Fisher exact test. The continuous variables were analyzed using *t*-test for independent samples and Mann-Whitney U test. Analysis of variance/multivariate analysis of variance (ANOVA/MANOVA) was used to determine the impact of the interaction of male smoking status and female age on certain numerical parameters. Binary logistic regression analysis was used to study the effect of male smoking on clinical pregnancy and delivery after controlling for potential confounding variables. Factors entering in the model were maternal age and smoking status, asthenozoospermia. Significant differences were considered to be values of $p < 0.05$.

RESULTS

Descriptive statistics of our results in 702 men, showed that 329 (46.9%) were nonsmokers and 373 (53.1%) were smokers. The average age of nonsmoking men (36.5 ± 5.2) was significantly higher than the average age of smokers (35.1 ± 4.5). The average age of female in the nonsmoking men group (31.5 ± 3.9) was significantly lower than the average female age in male smokers group (32.2 ± 4.4). There was no significant difference between the two groups considering the number of female smokers number and body mass index (BMI) (Table 1). Male smoking had no significant influence for all embryologic parameters above (Table 2).

In our results, we show that generally male smoking had no negative influence on clinical pregnancy rate, but early pregnancy loss rates were significantly more prevalent in the group of male smokers, thus, ongoing pregnancy rate and live birth rate was significantly lower in the group of male smokers (Table 3).

Logistic regression analysis was performed to determine if male smoking leads to unsuccessful clinical pregnancy in all age groups of women. Male smoking had no significant influence in all age groups of women for not achieving clinical pregnancy (Table 4).

Male smoking significantly increases the chances of the couple not achieving a live birth 1.56 times [95% confidence interval (95% CI) (1.15-2.12)]. Women older than 35 years in the group of male smokers had 2.42 times

Table 1. Demographic characteristics of patients in relation to male smoking status.

Parameters	Male Non Smokers	Male Smokers	p Value
Number of patients	329 (46.87%)	373 (53.13%)	
Males (age) (mean±SD)	36.5±5.2	35.1±4.5	0.00015 ^a
Females (age) (mean±SD)	31.5±3.9	32.2±4.4	0.027 ^a
Number of Female smokers (%)	117 (35.56%)	132 (35.39%)	0.96
Female BMI (mean±SD)	24.3±4.05	24.6±3.90	0.36

BMI: body mass index.

^a A p value of <0.05 was considered to be statistically significant.

Table 2. Influence of male smoking on embryologic parameters.

Male Variables (mean±SD)	Male Non Smokers	Male Smokers	Mann-Whitney U Test	p Value ^a
Number of retrieved oocytes (median)	10.8±6.6 (10)	11.1±6.9 (10)	Z=0.17	0.86
Number of mature oocytes (median)	9.0±5.7 (8)	9.2±5.9 (8)	Z=0.02	0.98
Number of fertilized oocytes (median)	6.8±4.2 (6)	6.8±4.5 (6)	Z=0.4	0.67
Fertilization rate	0.8±0.2	0.8±0.2	Z=0.19	0.85
Number of excellent quality transferred embryos (median)	2.3±0.7 (2)	2.3±0.8 (3)	Z=0.9	0.35
Number of transferred embryos (median)	2.6±0.6 (3)	2.6±0.7 (3)	Z=0.2	0.8

Z: Mann-Whitney U test.

^a Significant p value.

Table 3. Clinical results in correlation to smoking status of male patients of all ages per embryo transfer.

Clinical Parameters	Non Smokers n (%) ^a	Smokers n (%) ^b
Pregnancy rate	173 (52.6)	192 (51.5)
Clinical pregnancy rate	160 (48.6)	164 (43.9)
Biochemical pregnancy rate	13 (7.69)	28 (13.4)
Ongoing pregnancy rate	151 (45.9)	134 (35.9) ^c
Live birth rate	150 (45.6)	131 (35.1) ^c
Early pregnancy loss rate	9 (5.63)	30 (18.3) ^c

^a In the group of non smokers we had one patient with late pregnancy loss at 18 weeks of pregnancy.

^b In the group of smokers, we had two patients with late pregnancy loss, one at 15 weeks of pregnancy and one at 17 weeks of pregnancy. We also had one patient with deliver a fetus mortus *in utero* at 25 weeks of pregnancy.

^c A p value of <0.05 was considered to be statistically significant.

Table 4. Binary logistic regression: Overall relative chance of clinical pregnancy in IVF process, according to male smoking status.

Women's Age	Male Smokers	n	OR (crude)	OR (adjusted for athernozoospermia)	OR (adjusted for female smokers)
All ages	Non smokers	329	1.21 (0.89-1.62)	1.22 (0.91-1.64)	1.201 (0.90-1.62)
	Smokers	373			
Women <35 years	Non smokers	239	0.96 (0.67-1.37)	0.99 (0.69-1.42)	0.96 (0.67-1.37)
	Smokers	234			
Women >35 years	Non smokers	90	1.69 (0.97-2.93)	1.63 (0.93-2.85)	1.34 (0.75-2.37)
	Smokers	139			

OR: odds ratio.

[95% CI (1.33-4.41)] higher risk for not achieving a live birth. The interaction of female age and male smoking in a logistic predictive model showed that female age and male smoking were significant risk factors, namely predictors,

for not achieving a live birth. (Table5). In this study, we analyzed both men and women side-stream (SS) smoke exposure compare to live birth rate (Table 6). Results indicated that men exposed to SS smoking do not have a

Table 5. Binary logistic regression: Overall relative chance of live birth in IVF process according to male smoking status.

Women's Age	Male Smokers	n	OR (crude)	OR (adjusted for athernozoospermia)	OR (adjusted for female smokers)
All ages	Non smokers	329	1.53 (1.13-2.07)	1.56 (1.15-2.12)	1.53 (1.13-2.07)
	Smokers	373			
Women <35 years	Non smokers	239	1.15 (0.81-1.66)	1.21 (0.84-1.75)	1.16 (0.81-1.66)
	Smokers	234			
Women >35 years	Non smokers	90	2.52 (1.39-4.56)	2.42 (1.33-4.41)	2.53 (1.40-4.59)
	Smokers	139			

OR: odds ratio.

Dependent variable: non parturient/parturient OR.

Table 6. Binary logistic regression: Overall relative chance of live birth in IVF process according to male passive smoking status.

Women's Age	Male Smokers	n	OR (crude)	OR (adjusted for athernozoospermia)
All ages	Non smokers (both partners)	212	1.302 (0.828-2.047)	1.260 (0.798-1.991)
	Male passive smokers	117		
All ages	Non smokers (both partners)	212	1.543 (1.441-1.653)	1.599 (1.492-1.714)
	Female passive smokers	241		

OR: odds ratio.

Dependent variable: non parturient/parturient OR.

Table 7. Binary logistic regression model: Overall relative chance of live birth in ICSI process, according to male smoking status and age of the female.

Variable	B	SE	Wald	Significant p Value
Women (<35 years ^a / ^{>} 35 years)	0.82	0.17	21.8	0.000
Men (non smokers ^b /smokers)	0.36	0.16	5.23	0.022
Constant	-0.06	0.12	0.27	0.601

B: coefficient; SE: standard error; Wald: Wald test; Sig.: significant p value.

Dependent variable: non parturient/parturient OR (odds ratio).

^a Referent category: age/women <35 years.

^b Referent category: non smoker.

significant influence on the live birth rate in either of the women's age groups. After the adjustment for asthenozoospermia, male SS smoking was again confirmed to be an insignificant predictor for a live birth delivery. Compared to these results, women exposed to SS smoking were confirmed as a significant predictor for unsuccessful live birth delivery 15.4 [95% CI (1.44-1.65)]. Finally, the values of coefficient B suggest that from the two analyzed factors, the age of the female is a more significant factor for not achieving a live birth (B = 0.82) than male smoking (B = 0.36) (Table 7).

DISCUSSION

It is challenging to elucidate the role of male smoking upon the results of assisted reproduction technique (ART). One of the first studies that investigated the effect of paternal smoking on reproduction concluded negative influence on conception and decrease the chance of female partner to achieve live birth in ART [19]. Few studies that

followed, confirmed negative impact of paternal smoking on clinical pregnancy rate [20,21].

Our study shows that male smoking does not have a negative impact on the clinical pregnancy rate. These findings support the observations of ICSI as a preferred fertilization method because it bypasses the deficiency of natural IVF where spermatozoa are unable to penetrate the ovum [22].

It is known that the women's age is a strong influence factor on success of ART [23]. The advanced age of the women is connected with a low live birth rate [24]. Our study confirms that the age of the woman had a significant impact on all parameters that result from the IVF process. At all women's ages, male smoking signifies an important factor for the loss of an ongoing pregnancy rate and live birth rate. The interaction of female age and male smoking in a logistic predictive model that does not achieve a live birth showed that both smoking of men and age of the women are important risk factors, namely predictors, for not achieving a live birth. Comparison between the

effects of male smoking and female age on lower live birth rates suggests that female age has a stronger negative impact on live birth rates. Spermatozoa of men with severe DNA sperm fragmentation (smoking is associated with that cause) are still able to produce normal pronucleus formation following ICSI [25]. However, the advanced age of the women brings low quantity and quality oocytes and cleavage, stage embryos, that are not capable of repairing a certain degree of DNA sperm damage [26], resulting in a high rate of early pregnancy loss [26-28].

Our study detected that male smoking compromised the quality of the early pregnancy. Significantly higher early miscarriage rates were observed in men smokers resulting in lower ongoing pregnancy rate in this group of patients. This situation influenced the overall result of the IVF process and live birth rate. Namely, for all female age groups, male smoking represented a significant negative factor for achieving a live birth, but interaction of women's age more than 35 years and male smoking increased the risk for not achieving a live birth for 2.42 times 95% CI (1.33-4.41).

It is very important to understand the influence of SS smoking exposure compared to main-stream smoking effect on the fertility results. In his study, Neal *et al.* [29] concluded conclude that the reproductive consequences of SS smoking are as great as those observed in active smokers [29]. In our study, analyzed patients were exposed to household smoking, because smoking in public places is prohibited in our country.

We analyzed both men and women SS smoke exposure compared to live birth rates. Results indicated that men exposed to SS smoking do not have a significant influence on the live birth rate in all the women's age groups. Compared to these results, women exposed to SS smoking were confirmed to be significant predictors for unsuccessful live birth rates. We speculate that this association between women SS smoking exposure and decrease rate of live births is likely due to the results of spermatozoa DNA damage that occurred in the pre conception period. It is also very important to comment that all smokers are not infertile, which suggests that genetic variations or poly-morphisms in DNA repair, apoptosis, and xenobiotic metabolism genes in smokers may increase susceptibility to infertility [30].

The outcome of IVF cycles is dependent on many factors, primarily ranging from the age of the patients, ovarian reserve, endocrine status of the patients, BMI, more elaborate conditions such as endometriosis, pelvic inflammatory disease, immunology and others. When analyzing a single effect over IVF, these conditions also need to be analyzed.

This study confirms the negative impact of male smoking on ART. Generally, in both male and female pa-

tients, the approach to quit smoking and taking antioxidants, can improve the reproduction potential in both partners while trying to conceive the natural way or by ART.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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