

## Impact of Smoking on Sperm's DNA and Assisted Reproductive Techniques Outcome

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### Abstract

The effect of smoking on Assisted Reproductive Technology (ART) results in male was considered as a deleterious factor in pregnancy outcome among IVF patients. Male smoking is associated with lower success rate in ICSI and IVF couples. Smoking significantly decreased live birth rates and has a deleterious effect on sperm's DNA. This study was designed to evaluate the effect of male cigarettes smoking on the outcome of ICSI and IMSI, referring to clinical pregnancy, implantation and miscarriage rate. Therefore a total of 255 couples were diagnosed with severe oligo-astheno-teratozoospermia, 3 years of primary infertility, the woman aged 30 years or younger and an undetected female factor were randomized to IVF micro insemination treatments. Males were classified according to smoking into two major groups: group 1 (109) smokers and group 2 (146) non-smokers. The smoker group including (44 male) admitted for ICSI procedures and (65 male) admitted for IMSI procedure. While, non-smoker group included (76 male) and (70 male) were admitted for ICSI and IMSI respectively. A comparison between the two groups was based in terms of pregnancy, miscarriage and implantation rates. Student t-test was adopted to assess the significant differences between the two groups. The results of our study showed no significant difference in clinical pregnancy, implantation and miscarriage rate between smoker and non-smokers men. While, the difference was high significance in clinical pregnancy and implantation in IMSI group when compared to ICSI group and lowest significance difference was recorded in miscarriage rate in IMSI group when compared to ICSI group. In conclusion this study shaded more light on the effect of cigarettes smoking on male fertility and the cutting edge procedures used to solve the issue of frequent ICSI failure in the infertile couples. It also showed that clinical pregnancy and implantation rates are increased and miscarriage rate decreased in non- smoker couples male when IMSI procedure was used for them.

**Keywords:** Male infertility, Sperm DNA, Cigarettes smoking, IMSI, ICSI

### 1. Introduction

It is well known that Cigarettes smoking has adverse effect on all vital activities and since more than ten decades scientists of reproduction published several publications concerning risk of cigarettes smoking on fertility and conception as well as most phases of the development of the child in the womb and on post-natal survival (1).

Thus, smokers are 3.4 times more likely to take more than a year to conceive and also have two thirds the chance of conceiving compared with non-smokers (2). In animal models, exposure to tobacco compounds adversely affects pre and post-implantation embryo developmental competence (3-5).

In 2008, the American Society of Reproductive Medicine reported some of the negative reproductive consequences associated with smoking include: quicker depletion of ovarian follicles, conception delay, increased risk of spontaneous miscarriage in

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both natural and assisted conception cycles, and increased risk of birth defects (6).

Smoking still compromise reproduction and success rates of assisted reproductive technology (7-8). It was found that tobacco component like nicotine, cadmium and poly-aromatic hydrocarbons on has direct effects on estrogen synthesis and metabolism (9). As well as reduction of ovarian reserve (10-12) and induces fertilization failures (13, 8).

Hazards of smoking are several and not only affect the fertilization capacity but also impair the genital system as it has the ability to alter uterine-Fallopian tube function, alteration of the meiotic spindle, leading to chromosome errors and also acceleration of follicular depletion. Menopause occurs 1-4 years earlier in women who smoke and an increased incidence of Premature Ovarian Failure (POF) with remarkable increase in FSH levels reached 60-70 % higher which represent 40% of active smokers than non-smokers (10,14-19).

The natural conception rate in couples with a smoking male partner demonstrate a significant reduction in fecundity, with an increased time-to-pregnancy, only when tobacco consumption is above 15 cigarettes a day (20-21).

Couples with a male smoker were reported to have reduced fertilization rate, implantation rate, pregnancy rate and a reduced probability of achieving a 12-week gestation so that the identification of a number of abnormalities in the genetic material of spermatozoa produced by smokers indicate that, even if Intracytoplasmic sperm injection (ICSI) overcomes many of the drawbacks in sperm function determined by tobacco exposure, the presence of DNA adducts, DNA fragmentation and may be an increased aneuploidy rate are likely to impair IVF cycle prognosis. Indeed, a significantly lower pregnancy rate with either conventional In Vitro fertilization (IVF) or (ICSI) was reported in couples with a smoking husband (22-24).

Because smoking exerts oxidative stress, sperm DNA may also experience damage. This has been demonstrated in various studies (25), and altered DNA from smoking fathers has been detected in embryos, the number of cigarettes consumed being associated with benzopyrene-DNA adducts (19). So, this study was aimed to evaluate the impact of smoking on the integrity of sperm's DNA and fertilization outcome using Intracytoplasmic sperm injection (ICSI) and Intracytoplasmic Morphologically Selected Sperm Injection (IMSI). Also, to figure out which technique, ICSI or IMSI, is better to get high fertilization and implantation rates

and low miscarriage rates in smoker and non-smokers male couples.

## **2. Materials and methods**

### **2.1. Patient selection**

The study subjects were 255 couples (255 cycles) enrolled in the ICSI program in the Centre for Human Reproduction, Dr. Fairs Medical Center. Indication Criteria included: (I) Male factor infertility, (II) The woman being 40 years or younger, (III) At least 8 months of marriage without normal pregnancy.

The male factor infertility was defined according to WHO/Kruger criteria for sperm concentration, motility and morphology, which have previously been tested. If at least two of these three parameters were abnormal, the couples were considered for ICSI treatment. Written consent was taken from all the patients and the study was performed according to the norms of the Institutional Ethics Committee.

All study participants were blinded to treatment assignment for the duration of the study. The following groups were formed: male smoker (n = 109; group 1) and non-smokers male (n = 146; group 2). Although 120 couple enrolled to ICSI technique and 135 couple enrolled to IMSI technique.

### **2.2. Ovarian stimulation**

All patients were submitted to the same scheme of controlled ovarian stimulation. After pituitary down regulation with Decapeptyl (Triptorelin) - 0.1mg/day (United Pharmacies-UK) started in the mid-luteal phase, recombinant human FSH (r-FSH/Gonal F1; Serono, SP, Brazil) was administered at a starting dose of 150-300 IU, depending on the age of the patient an recombinant LH (r-LH/Luveris1, Serono, SP, Brazil) was administered at a dose of 75 IU/day for a period of 7 days. On day 8 of stimulation, follicular development was monitored by 7 MHz transvaginal ultrasound only (Medison Digital Color MT, Medison Co. Ltd, Seoul, Korea) and the FSH dose was adapted according to ovarian response. The r-LH supplementation was increased to 150 IU/day when one or more follicles measuring 10 mm in diameter were found. When at least three follicles measuring 17 mm in diameter were observed, recombinant chorionic gonadotropin (HCG/Ovidrel1 250 mg Serono, SP, Brazil) was administered. Transvaginal, ultrasound-guided oocyte retrieval was performed 36 h after HCG.

### **2.3. Semen Preparation**

Fresh ejaculated semen was prepared for IVF by discontinuous concentration gradient.

#### 2.4. Egg retrieval

Transvaginal ultrasounds was carried out and oocytes were aspirated from follicles in both ovaries through a needle that is used to pierce the vaginal wall and puncture the follicle. The retrieved oocytes were placed in culture dishes containing culture medium (G-IVF plus with HAS, Vitrolife, Gotebrog, Sweden) and covered with paraffin oil (Light mineral oil, Irvine Scientific, Santa Ana, CA) and kept in an incubator (Labotect C200) that is maintained at 37° of 5–6% CO<sub>2</sub> in humidified air.

#### 2.5 Removal of cumulus cells: (Denudation)

The surrounding cumulus cells were removed after exposure to a HEPES buffered medium containing hyaluronidase (80 IU/mL, Irvine Scientific, Santa Ana, CA,). Only oocytes that had reached metaphase of the second meiotic division (MII) were selected for injection.

#### 2.6. IMSI procedure

One ml aliquot of sperm cell suspension was transferred to a 5 ml micro droplet of modified HTF medium containing 10% polyvinyl pyrrolidone solution containing 5% human serum albumin (PVP medium Irvine Scientific, USA). This microdroplet was placed in a sterile glass dish (FluoroDishwellcon BV Netherland-Amestrdam) under sterile paraffin oil (Light mineral oil, Irvine USA). The sperm cells, suspended in the micro droplet, were placed on a microscope stage above an Upland Above 100 oil/1.35 objective lens previously covered by a droplet of immersion oil. In this manner, suspended motile sperm cells in the observation droplet could be examined at high magnification. The spermatozoa used for IMSI were classified in to 5 groups. Grade I consisted of spermatozoa free of any morphological abnormality (Normal spermatozoa). A spermatozoon was classified as morphologically normal when it exhibited a normal Nucleus as well as acrosome, post-acrosomal lamina, neck, tail and mitochondria, besides not presenting a cytoplasmic droplet or cytoplasm around the head.

For the nucleus, the morphological state was defined by the form and content of the chromatin. The criterion for normality of nuclear form was a smooth, symmetric and oval configuration. Normal means for length and width were estimated the nuclear and no vacuoles inside the heads form was considered abnormal if extrusion or invagination of the nuclear chromatin mass has been detected (regional malformation of nuclear form or DNA fragmentation). Chromatin content was considered abnormal if one or more vacuoles were observed to

occupy more than 4% of the nuclear area. A nucleus was considered normal if both nuclear form and chromatin content were normal. When no Grade I spermatozoa were available or the number was insufficient for injection, spermatozoa were classified by head forms as Grade II: large oval: (5.31 mm), small oval (4.19 mm), wide (>3.7 mm width) or narrow (<2.9 mm width); Grade III: presence of regional disorders; Grade IV: presence of large vacuoles on 5–50% of head surface and Grade V: presence of large vacuoles on >50% of head surface.

After the sperm selection, the microinjections were carried out in the same manner as in ICSI. Spermatozoids were still motile when captured for final selection.

#### 2.7. Fertilization

The assessment of fertilization was carried out about 17 h after injection using Nomarski differential interference contrast optics.

#### 2.8. Embryos Transfer

Embryos transferred on day 3 or 5 after egg retrieval according to the embryo quality day 3 if the quality of embryos is grade three or four and transferred on day 5 if quality of embryos is grade one or two.

#### 2.9. Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer. All women with a positive test had a trans-vaginal ultrasound scan 2 weeks after the positive test. Clinical pregnancy was defined as a positive  $\beta$ -human chorionic gonadotrophin ( $\beta$ -HCG) assay and the presence of at least one gestational sac with fetal heartbeat detection by trans-vaginal ultrasound examination. Pregnancy rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

#### 2.10. Statistical analysis

Our data were analyzed using statistical package of social science SPSS (version 18.0). Student T test was used to study different age, retrieved oocytes, injected oocytes, fertilized, embryos transferred, between ICSI and IMSI groups. While Chi-square test was used to compare, clinical pregnancy, implantation and miscarriage rates between ICSI and IMSI groups.

### 3. Results

Table (1) showed there was significant difference in clinical pregnancy and implantation but there is no significant difference in miscarriage rate between

smoker and non-smokers men results from normal sperms without DNA fragmentation. While, in table (2) there was no significant difference in clinical pregnancy and implantation between male smokers in ICSI and IMSI group on the other hand there is significant difference in miscarriage rate between male smoker and non-smoker in ICSI and IMSI technique, more over when we compare clinical pregnancy, implantation and miscarriage between ICSI and IMSI technique in non-smokers male to select less fragmented sperms there was high significance difference in clinical pregnancy and implantation in IMSI group than ICSI group and lower significance difference in miscarriage rate in IMSI group than ICSI group.

**Table 1. Comparison of clinical pregnancy, Implantation and miscarriage rates arising from Two Groups smokers and non-smoker's men's**

	Male smokers n= 109	Male non-smokers, n=146
Clinical Pregnancy %	47.7% (52/109)*	58.2% ( 85/146)*
Implantation %	40.4% (44/109)*	49.3% ( 79/146)*
Miscarriage %	17.3% ( 9/52)	21.2% ( 18/85)

\* Significant difference at  $P > 0.05$

**Table 2. Comparison of clinical pregnancy, implantation and miscarriage rates in ICSI and IMSI Group according to arising from subgroups Smoker and non- smoker men's.**

		ICSI	IMSI
Male Smoker	Pregnancy	45.4% (20/44)	49.2% (32/65)
	Implantation	40.9% (18/44)	40% (26/65)
	Miscarriage	30% (6/20)*	9.4% (3/32)*
Male non-Smoker	Pregnancy	44.7% (34/76)**	72.8%(51/70)**
	Implantation	30.3% (33/76)**	70%(49/70)**
	Miscarriage	32.4% (11/34) *	13.7% (7/51)*

\* Significant difference at  $P > 0.05$

\*\* High significant difference  $P > 0.01$

#### 4. Discussion

Due to the fact that cigarette smoke contains more than 4000 harmful substances, there has long been concern that smoking could have adverse effects on

male reproduction. First and foremost, tobacco smoking leads to reduced semen quality including semen volume, sperm density, motility, viability and normal morphology in smokers. Furthermore, reproductive hormone system disorders, dysfunction of spermatogenesis, sperm maturation process and impaired spermatozoa function have also been observed in smokers. Despite the various harmful effects of smoking on male fertility, most male smokers are still fertile but have higher risk of sub-fertility or infertility. Cigarette smoking is widely believed to be associated with decreased fecundity in naturally conceiving populations; Smoking has been found to have an adverse effect on fertility and conception as well as most phases of the development of the child in the womb and on post-natal survival (1). However, the effect of female smoking on pregnancy outcomes in patients undergoing IVF is unclear.

This result showed significant difference in clinical pregnancy, implantation rate between smoker and non-smokers men and lowest significance difference was recorded in miscarriage rate between IMSI and ICSI group in smoker men. While, the difference was high significance in clinical pregnancy and implantation in IMSI group when compared to ICSI group and lowest significance difference was recorded in miscarriage rate in IMSI group when compared to ICSI group in non-smokers men. Several authors attributed the decrease in male fertility to the cigarettes smoking which contain a lot of cytotoxic and genotoxic compounds nicotine, tar, carbonic monoxide (CO), polycyclic aromatic hydrocarbons (PAHs), radioactive substances and heavy metals which are closely related to male fertility according to the researches in recent years (26).

*In vitro* study found that nicotine concentration decreased the sperm motion and viability parameters significantly (27). Nicotine reduced the percentage of viable spermatozoa and promoted spermatozoa apoptosis with DNA fragmentation or altered chromatin compactness. This study indicated that nicotine may be considered as a toxic component of tobacco smoke that directly impairs male reproductive functions. Other studies revealed that nicotine could induce Leydig cell apoptosis and inhibit androgen biosynthesis in Leydig cell, suggesting the possibility that nicotine may impair male reproductive hormone system (28, 29).

Our study in agreement with studies of (1, 23, 31) who reported that smoking has a negative impact on the reproduction of a couple and smoking by male partners significantly decreases the success rates of assisted reproduction procedures, not only of IVF,

but also of ICSI.

Cigarettes smoking induce sperm DNA fragmentation and the percentage of sperm with DNA fragmentation is higher in smokers (12) and repair capacity is significantly decreased in ejaculated spermatozoa, genetic damage is passed on to the embryo (31). Smoking by male and/or female partners has been reported to compromise reproduction and success rates of assisted reproductive technology (8, 32).

Apart from putative adverse effects during fertilization, altered DNA in spermatozoa may hamper the development of the embryo and the present results are agreed with Dai *et al.*, 2015 they observed that, the process of fertilization involves a sperm fusing with an ovum, in addition to the balance of ROS and antioxidants in semen plasma, sperm and testis plays an important role in maintaining the normal function of spermatozoa because of their high susceptibility to oxidative stress (33). Since sperm lipid and DNA are vulnerable to ROS, increased ROS level will lead to DNA fragmentation and apoptosis which will result in the impairment of sperm function leading to lower male fertility.

The present results showed significant difference between ICSI and IMSI technique in clinical pregnancy and implantation rate between male smokers in ICSI and IMSI group on the other hand there was high significance difference in clinical pregnancy and implantation in IMSI group than ICSI group on ( $P < 0.01$ ) and lower significance difference in miscarriage rate in IMSI group than ICSI group ( $P < 0.05$ ) in non-smokers male.

Smoking has been found to have an adverse effect on fertility and conception as well as most phases of the development of the child in the womb and on post-natal survival (1). Smoking by male partners significantly decreases the success rates of assisted reproduction procedures, not only of IVF, but also of ICSI. Apart from putative adverse effects during fertilization, altered DNA in spermatozoa may hamper the development of the embryo (23).

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Intracytoplasmic sperm injection (ICSI) overcomes many of the draw backs in sperm function determined by tobacco exposure, the presence of

DNA adducts, DNA fragmentation and may be an increased aneuploidy rate are likely to impair IVF cycle prognosis; Indeed, a significantly lower pregnancy rate with either conventional In Vitro fertilization (IVF) or (ICSI) was reported in couples with a smoking husband (22-24).

In conclusion, this study shaded more light on the effect of cigarettes smoking on male fertility and the cutting edge procedures used to solve the issue of frequent ICSI failure in the infertile couples

It also, showed high magnification in the IMSI method reveals critical details in sperm morphology, especially that of the nucleus, allowing selection of heights quality DNA of sperms for ICSI.

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