Original Article

Maternal Exposure to Second-hand Smoke and Super Ovulation Outcome for Assisted Reproduction

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Abstract

Background: The effect of smoking cigarettes is followed by diverse effects on ovaries and developing follicles but the effect of passive smoking on ovarian function is unknown. On the other hand, the ovarian response to induction is a very important step in assisted reproduction. The aim of this study is to compare ovarian response in passive smokers and non-passive smokers in an assisted reproductive program.

Materials and Methods: In a cohort study at the Isfahan Fertility and Infertility Center, there were 72 women in the passive smoker group and 72 women in the non-passive smoker group who underwent an assisted reproductive technology (ART) program. The follicle number at administration of human chorionic gonadotropin (HCG), number of gonadotropin ampoules and duration of super ovulation induction were compared.

Results: Statistical analyses indicated that the number of mature follicles in the passive smoker group was not different from the control group; but the number of unresponsive cycles to super ovulation in the passive smoker group (33.3%) was significantly higher than the control group (12.5%). Duration of induction and number of gonadotropin ampoules were not different between the two groups.

Conclusion: The results of this study show that exposure to second-hand smoke increases the chance of unresponsiveness to ovulation induction. This condition may be due to the result of decline in ovarian reserve in second-hand smokers. The duration of induction and number of gonadotropin ampules is similar in the two groups. Furthermore, the results show that exposure to cigarette smoke does not clinically impact women with good ovarian reserve.

Keywords: Passive Smoking, Super Ovulation, Assisted Reproductive Technology

Introduction

Cigarette smoking is one of the factors harming human health and is the target of many epidemiological studies in reproductive health. The results of these studies, mainly focusing on infertile couples who underwent assisted reproductive technology (ART), indicate the harmful effects of active smoking on reproductivity. According to these studies, failure of fertilization, implantation and cleavage are higher in smokers (1-3).

In addition these studies show that metabolites produced by cigarette smoke, such as cadmium and cotinine, increase oxidative stress in follicular fluid (4). Also, the existence of these metabolites along with benzo [a] pyrene-DNA in the corpus luteum and ovarian vessels directly affect the ovarian steroidogenesis and ovarian gametogenesis (5, 6).

However, there are few studies on the effects of exposure to cigarette smoke on the ovarian function of non-smokers. According to the US National Health and Nourishment Report, investigations on cotinine and biomarkers have shown that 50% of American non-smokers are exposed to cigarette smoke (7). In Iran, 39.75% of non-smoker women are exposed to cigarette smoke and a study in Tehran showed that 24.5% of women have been exposed to cigarette smoke for more than 19 years (8). The amount of cigarette metabolites in the serum of non-smoker women who were exposed to cigarette smoke is 30% to 40% of heavy smoker women (9).

A study on animals which have been exposed to cigarette smoke shows that cigarette smoking metabolites target developing primary follicles as well as resting ones (10). The Socio-medical Society of Fertility also reported that exposure to cigarette smoke has harmful effects on the ovaries, the func-

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tion of the reproduction system and causes early menopause (11).
It is also reported that women's exposure to cigarette smoke increases the risk of delay in pregnancy for more than six months, reduces the number of follicles and the release of estrogen and arumatizasion (1).
However, studies about the effects of exposure to cigarette smoking on the ovarian response of non-smoker women have been controversial. Some researchers reported that women who were exposed to cigarette smoking required more gonadotropin for ovarian response and they had a lower number of mature oocytes in the ovarian induction process (2, 3, 12, 13). In contrast, Wright et al. did not show any significant difference between the peak serum of estradiol and the number of oocytes in women exposed to cigarette smoke (14).
A lack of sufficient studies to clarify the effects of exposure to second-hand smoke on women's fertility potential and the controversial results in the literature; in addition to the importance of appropriate ovarian response to stimulation during ART and a better understanding of the effects of environmental factors such as exposure to second-hand smoke was the basis for this study. Therefore, this study aims to compare the ovarian response to stimulation in a group of women exposed to cigarette smoking and a control group. Thus, the number of mature follicles, number of gonadotropin ampoules necessary for ovarian response and the duration of ovarian stimulation in infertile women undergoing ART treatments were measured.

Materials and Methods

This study was carried out from Spring, 2007 to Summer, 2008 in the Isfahan Fertility and Infertility Center. This study is a double cohort study on 144 women: 72 in the group exposed to cigarette smoke and 72 controls. The study population included all women less than 40 years of age who were candidates for *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) due to primary infertility and were treated using gonadotropin for ovarian stimulation. All subjects used gonadotropin-releasing hormone (GNRH) agonists for ovarian down-regulation before ovarian stimulation. The entry criteria for the group exposed to cigarette smoking included at least one exposure in a closed area during the past two menstruations and during the treatment period. The control group included women who were not exposed to cigarette smoke. Individuals with ovarian endometriosis and endometrioma, systemic diseases and who were using anti-depression drugs were excluded. Women who stopped the treatment for reasons other than no ovarian response and ovarian hyper-stimulation were excluded.
Sampling method was simple. All women candidates of IVF and ICSI who had received gonadotropin for ovarian stimulation and were referring to the clinics for the first time after the stimulation for ultrasound assessment were interviewed. If they met the entry criteria and signed the consent form, they were entered into the study. Study approved by the Institutional Review Board of Isfahan University of Medical Sciences.
Data were collected using a questionnaire that included demographic data and the patients' IVF and ICSI outcomes. The questionnaire was completed by measuring height and weight to calculate body mass index (BMI), an interview with the subjects and data gathered from their medical files. Data related to the results of ovarian stimulation was collected from ultrasound results during the period of ovarian stimulation and at the time of HCG injection. The number of mature follicles (follicles with a diameter equal or greater than 16 mm) as well as the number of gonadotropin administered were recorded. The time distance between the first gonadotropin administrations to HCG injection was also recorded.
Data were analyzed using SPSS software and inferential statistical methods of chi square and independent t test.

Results

The findings show that the two groups have no significant difference in age, BMI and cause of infertility (Tables 1 and 2).

Table 1: Individual characteristics in the two groups

	Non passive smokers	Passive smokers	P value
Age (years)	28.1 (± 4.9)	28.2 (± 4.8)	ns
BMI (kg/m²)	25.2 (± 3.9)	25.2 (± 3.3)	ns

NS= No Signification

Most subjects were housewives (86.1% in the group exposed to cigarette smoking and 88.8% in

Table 2: Causes of infertility in the two groups

Cause of Infertility group	Male f.	Ovarian f.	Tubal f.	Unexplained
Passive smokers	45.8%	44.5%	6.9%	2.8%
Non-passive smokers	47.2%	43.1%	6.9%	2.8%

the control group).
There were 32% and 35.5% of the exposed group and control groups who underwent IVF while 78% and 64.5% underwent ICSI, respectively.
The number of gonadotropin ampoules follicle stimulating hormone (FSH) human menopausal gonadotropin (HMG) injected for ovarian stimulation in the two groups were not significantly different [mean 7.19 ± 7.9 for the exposed group and 8.19 ± 7.12 for the non-exposed group (t = 0.02)]. But the number of mature follicles in the exposed group was significantly different from the non-exposed group (mean 3.3 ± 7.3 and 7.4 ± 1.4, respectively, p = 0.02, t = 2.22).
In the statistical analysis, after omitting unresponsive cases to ovarian stimulation, the number of mature follicles in the exposed group with a mean of 4.58 ± 3.72 was not significantly different from the non -exposed group with a mean of 5.09 ± 3.68 (t = 0.73).
Those patients who did not respond to ovarian stimulation in the exposed group and the non-exposed group were 3.33% and 4.12% respectively and a comparison between the two groups showed a significant difference (p = 0.001, χ^2 = 10.89). Women exposed to cigarette smoke were 3.5 times more likely to not respond to ovarian stimulation than those who were not exposed. The time difference between the first day of last menstruation in the treatment cycle to receiving the ovule was a mean of 9.13 ± 2.4 days for the exposed group and 9.12 ± 1.9 days for the not-exposed group. The difference was not significant (t = 0.38).

Discussion

Previous studies on the extent of cigarette smoking components and its metabolites in the follicular fluid of women exposed to cigarette smoking have explained to some extent the harmful effects of smoking and exposure to cigarette smoking for non-smokers (4-6, 9). These studies show that environmental pollutants passing through the follicle's permeable membrane have reverse effects on the process of steroidogenesis on the ovary, can impose harmful effects on the women's fertility (9, 15) and disrupt the clinical effects of the ovarian stimulation process. However, the findings of this study showed no significant difference in the ovarian response, the number of gonadotropin ampoules and the duration of ovarian stimulation and was consistent with other studies (16, 17). The rate of ovarian non-response to ovarian stimulation in women exposed to cigarette smoking was higher, which shows the reverse effect of cigarette smoking on ovarian function. Similar to a report by Hillary, this result shows that the number of mature follicles after ovarian stimulation in women exposed to cigarette smoking was less than the controls (18, 19), but in later data analysis and after omitting the cases who didn't respond to ovarian stimulation, this difference was not significant any more. These results show that cigarette smoking has no effect on follicular growth *in vivo*. Even though the duration of exposure to cigarette smoking is not investigated in this study, chronic exposure to cigarette smoking probably is associated with the decrease of ovarian reserves and lack of ovarian response to stimulation.
In vitro studies on animals show that direct contact with polyaromatic hydrocarbons (PAH) produced by cigarette smoke targets primary and growing follicles as well as resting follicles, destroys ovarian follicles and decreases its functioning. Exposure to these materials leads to an ovarian response in humans which activates cell death in the oocytes of resting follicles and granulosa cells (20). The toxins in cigarette smoking cause DNA destruction, granulosa and cumulus oophorus cell death (apoptosis). Cumulus oophorus cells have a vital role in occyte maturity and growth of ovarian follicles. They facilitate the transportation of nutrition in and out of the ovule, the secretion of outcellular matrix which is necessary for ovulation and transferring hormone signals. The products of cigarette smoking; including nicotine, cadmium and poly aromatic hydrocarbons, controls the aromatase activity in granulosa cells. Therefore, the latter report may account for some of the probable mechanisms which effect the growth of the follicle (2, 21).

Conclusion

Considering the results of the present study and other studies, it can be concluded that the harmful effects of frequent exposure to cigarette smoking on the process of reproductivity reduces the ovarian reserve and leads to a nonresponse to ovarian stimulation. However, the existence of these materials in follicular fluid, if not associated with the destruction of preanteral follicles, appears that they cannot clinically affect the process of follicle maturity. However, further investigation is needed to evaluate the effects of these products on oocyte quality and ART outcome.

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