

## **Short communication**

# **The relationship between passive smoking and ovarian response outcome in ART cycles**

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### **Abstract**

**Background:** Smoking has negative effects on reproductive process. Exposing to cigarette smoking (passive smoking) may exert some effects as the direct smoking.

**Objective:** The aim of this study was to evaluate the correlation between ovarian response and passive smoking in women who underwent ART cycles.

**Materials and Methods:** One hundred-sixty patients who underwent ICSI between 2000 and 2001 were studied in a prospective cohort study. The case group included women whose husbands smoked at least 5 cigarettes daily for 1 year or more. The control group included women with nonsmoking husbands. Women with high FSH level (>12 IU/ml) were excluded. Long standard protocol with GnRH agonist and HMG were used in all patients. In vitro fertilization and embryo transfer was carried out in a standard fashion.

**Results:** Eighty one women were in case group and 82 in control group. Ovarian response variables were not significantly different between two groups but there was a significant relation between passive smoking and fertilization (RR= 1.18, 95% CI: 1.07-1.31). However pregnancy rate was not significantly different between two groups. Moreover there were no significant differences between heavy and light smokers in ovarian response outcomes.

**Conclusion:** This study showed no correlation between ovarian response parameters and passive smoking in women underwent ART cycles, whereas fertilization rate is significantly lower in this group compared to control group. It may be related to sperm quality than oocytes. Assessment of nicotin in follicular fluid and cytogenetic evaluation of embryo before transfer are recommended for more information and confirmation.

**Key words:** *Passive smoking, Ovarian response, In vitro Fertilization outcome.*

### **Introduction**

Cigarettes contain more than 2000 hazardous substances, for example, hydrocyanic acid, arsenic and many likely carcinogens such as nitrosamines. A person smoking 10 cigarettes per day ingests 0.7 µg cadmium.

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Negative effects of smoking have been confirmed in general health. Likewise cigarette may negatively affect reproductive process. Several studies have shown the relation between smoking with reduced fertility, ectopic pregnancy, abortion, early menopause, poor pregnancy outcome and also with cervical cancer. In addition, both male and female smoking could play important role in success rate of infertility treatment including assisted reproduction treatment (ART) cycles (1).

Exposure to cigarette smoking (passive Smoking) may exert same effects as the direct smoking. For example, smoking one cigarette in a room with closed windows raises the formaldehyde content in the room ten fold which could be hazardous for the families of smoking person. Studies showed that passive smoking in childhood and adolescence doubles the risk of developing lung cancer as a nonsmoking adult (2). In our country, passive smoking in women is more prevalent than the smoking among women themselves. The aim of this study was to evaluate the effect of indirect smoking on ovarian response outcome during ART cycles in women who had smoker husbands.

### Materials and methods

One hundred-sixty three patients underwent intracytoplasmic sperm injection (ICSI) were studied. This prospective cohort study was approved by ethics committee of Royan institute and performed between 2000 and 2001. The case group included women whose husband smoked at least 5 cigarettes daily for 1 year or more. The control group included women with non-smoking husband. Both groups were selected to start the treatment cycle simultaneously. Women with high FSH level ( $>12$  IU/ml) were excluded. All patients received subcutaneous Buserelin (Suprefact, Hoechst, Germany) 0.5 mg daily from 21<sup>st</sup> day of menstrual cycle. After two weeks, when suppression was achieved as defined by ultrasound (endometrial thickness  $<6$ mm and inactive ovaries), HMG 150-225 IU (Pergonal, Organon, Holland) daily was initiated. Ovarian monitoring by vaginal ultrasound and serum estradiol ( $E_2$ ) determinations were started on day 5 of stimulation and then individually followed. HCG (Profasi HP, Serono, Italy) was administrated 10000 IU intramuscular when at least two follicles had

reached to 18 mm of size. Oocytes aspiration was performed 35-37 hours after HCG administration under vaginal ultrasonography. Fertilization was achieved through ICSI and embryo transfer was performed 48-72 hours after oocyte retrieval. Luteal phase was supported by Pregesterone (Daroupaksh, Iran) 100 mg intramuscular daily beginning on the day after oocyte retrieval. Clinical pregnancy was diagnosed if a gestation sac with embryonic heart activity was seen by transvaginal ultrasound 4-5 weeks after embryo transfer. Metaphase II oocyte defined as high quality oocytes (3). Vision of 2 pronuclei (2PN) 18-24 hours after injection defines as fertilization (4). There were some difficulties in data collection since some women had no exact information about husband smoking habits such as duration or the number, therefore those cases did not enter to the study.

### Statistical analysis

Results were shown as mean  $\pm$  SD (standard deviation). Student t test,  $X^2$  and Fisher exact test were used for analysis. P-value  $<0.05$  was considered as significant level.

### Results

In this study, 81 women were in case group and 82 in control group. The causes of infertility in case group were: male factor (49 cases), tubal (13 cases), unexplained (4 cases), multi factor (3 cases) and the remaining with other causes. In control group, patients had male factor 66, tubal 6, unexplained 2 and other causes 8 cases. Mean age in passive smoking women was significantly lower than the control group. However ovarian response variables were not significantly different between two groups (Table I).

**Table I.** Ovarian response outcome in passive smoking women and non smoking group.

Variable	Case group(n=81)	Control group(n=82)	p- value
Age (y)	30.2 $\pm$ 5.7	32.0 $\pm$ 5.7	0.048
Basal FSH (IU/ml)	8.8 $\pm$ 2.3	5.8 $\pm$ 2.8	NS
Basal LH (IU/ml)	6.0 $\pm$ 4.2	6.9 $\pm$ 7.2	NS
Stimulation days (n)	10.8 $\pm$ 1.9	10.9 $\pm$ 2.4	NS
HMG ampoules (n)	29.9 $\pm$ 11.2	30.0 $\pm$ 9.5	NS
Total number of oocytes (n)	6.9 $\pm$ 3.9	6.6 $\pm$ 4.9	NS
Metaphase II oocytes (n)	5.0 $\pm$ 3.3	5.3 $\pm$ 3.7	NS
Pregnancy rate (%)	12 (15%)	22 (27%)	NS

NS: Non Significant

With dividing total oocyte by fertilized and no fertilized and by calculation of relative risk and confidence interval 95% , there was a significant relation between passive smoking and fertilization (RR=1.18, 95% CI: 1.07-1.31). There was no significant difference between two groups in pregnancy rate.

According to the number and duration of smoking, two selected groups including heavy smokers (more than 10 cigarettes for more than 10 year) and light smokers (less than 10 cigarettes for less than 10 year) were compared in 5 variables of ovarian response outcomes. There were no significant differences between two groups in these parameters.

### Discussion

The influence of smoking on reproductive process and also ART cycle outcome have been shown in several studies although the results are still controversial. The purpose of this study was to assess the relation between cycle outcome of ART and passive smoking. Our data revealed no relation between ovarian response outcome and passive smoking, otherwise fertilization rate was significantly reduced in passive smokers. Study of Sharara et al (1994) showed that the female fertility may be affected by cigarette in smokers' women (5). The mechanisms by which smoking reduced female fertility are still controversial. However, several studies have shown that two main mechanisms in this view reduce ovarian reserve which resulted to reduce oocytes retrieved in ART cycle and also increase abortion rates (1).

On the other hand, some studies on number of oocytes in smoking women showed that total oocytes do not reduce although the quality of oocytes were not evaluated in those studies (6,7). Study on the oocyte quality showed that oocyte maturity in smokers' women which their follicular fluid contents nicotine was lower than the nonsmokers (6). In this study fertilization rate was used as an objective measure of oocyte maturity, since only mature oocyte were fertilized, and the results showed that fertilization rate were lower in smoker group and this reduction was significant in older women(6).

In the present study, no direct correlation has been observed between oocyte number and quality and also ovarian stimulation variables such as HMG ampouls and stimulation days, with passive smoking. Contrary, the study of Klonoff-cohen et al (2001) revealed that there is a 46% decrease in the number of total oocytes in women when men

smoked during ART cycles (8). However the only significant data in our study was fertilization rate which was lower in passive smokers, while this group was younger than the control group. Old age is a known factor to influence oocyte number and quality. Since the other variables of ovarian response were not different between two groups, decrease fertilization rates in passive smokers may be related to male factor. It means that fertilization rate could be affected by sperm abnormality. Moreover, male factor was the most frequent cause of infertility in both groups (49 patients in case group, 66 patients in control group) .Consequently, significant decrease of fertilization rate might be affected by cigarette influence. Furthermore, chemical components of tobacco smoke may induce alkylation of DNA both in the oocyte and sperm which resulting to abnormal cytogenesis embryo (1). Sun et al (1997) showed that at least one quarter of semen analysis have between 5-40 sperms with fragmented DNA(9) .The percent of DNA fragmentation is significantly higher in smoker than non smokers. In present study, the difference in mean age between two groups was near significant level (P=0.048).Nevertheless, age can not be confounder in this study because mean age was lower in case (Passive smoker) group, while fertilization and pregnancy rates were higher in control group who were older. This finding was in consistency with effect of age. Regarding to duration and number of cigarette smoking, there was no difference between heavy and light passive smokers in cycle outcomes in our study. Therefore, the negative effect of smoking probably starts from least doses as shown in previous studies (2).

### Conclusion

This study showed no correlation between ovarian response and passive smoking in women who underwent ART cycles whereas fertilization rate was significantly lower in this group compared to control group. In this view, assessment of nicotine in follicular fluid during oocyte aspiration and cytogenesis assessment of embryo before transfer by Preimplantation Genetic Diagnosis (PGD) is recommended for more information and confirmation.

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