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The effect of cotinine concentrations in seminal plasma and follicular fluid on the pregnancy outcomes of couples undergoing assisted reproductive techniques

Füsun TERZİOĞLU[1,*], Bensu KARAHALİL[2], Çiğdem YÜCEL[3], Rukiye TÜRK[4]

[1]Faculty of Health Sciences, İstinye University, İstanbul, Turkey
[2]Department of Toxicology, Faculty of Pharmacy, Gazi University, Ankara, Turkey
[3]Department of Obstetrics and Gynecology Nursing, Faculty of Nursing, Hacettepe University, Ankara, Turkey
[4]Department of Nursing, School of Health Sciences, Kafkas University, Kars, Turkey

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Background/aim: This study determined the effects of cotinine concentrations in follicular fluid (FF) and seminal plasma (SP) on the pregnancy outcome of couples using assisted reproductive techniques (ARTs).

Materials and methods: This study was conducted as a case-control study. A total of 217 couples were included in the study. Among these couples, there were nonsmokers (66 women and 40 men), passive smokers (106 women and 54 men), and active smokers (45 women and 123 men). Demographic and smoking data were collected by questionnaire at the onset of treatment. FF and SP samples were obtained from the couples on the day of oocyte retrieval.

Results: The cotinine concentrations in the FF and SP of nonsmokers were significantly lower than they were in the other groups ($P = 0.001$). The difference in cotinine concentrations detected in FF between women with positive pregnancy test results and women with negative pregnancy test results was statistically insignificant. It was also determined that the percentage of clinical pregnancy was lower in nonsmoker women than in passive smoker or smoker women ($P > 0.05$).

Conclusion: Although we found there was no significant difference in the pregnancy outcome between nonsmoker and passive smoker or smoker women, smoking cessation should be an integral part of ARTs.

Key words: Infertility, assisted reproductive techniques, seminal plasma, follicular fluid, cigarette, cotinine concentrations

1. Introduction

Tobacco is the only legal product that kills a large proportion of its consumers and is also the most preventable cause of death today. The World Health Organization estimates that tobacco use is currently responsible for almost 6 million deaths each year. It is also estimated that this number will reach 8 million by 2030 and more than 80% of tobacco-related deaths will occur in developing countries (1). Smoking is a major health problem in Turkey as a developing country. According to the 2010 Global Adult Tobacco Survey Turkey Report, approximately 16 million adults aged 15 years and older smoke (47.9% of men, 15.2% of women) and Turkey is among the 10 countries where two-thirds of the world's smokers live (2).

Besides the adverse effects of smoking on the general health of individuals, it can have negative effects on reproductive functions depending on the number of cigarettes smoked. It has been shown in many studies that smoking, as one of the main lifestyle-related risk factors, has negative effects on the reproductive functions of men and women and causes failure in the treatment process with assisted reproductive techniques (ARTs) (3–9). It has been also shown that being exposed to secondhand smoke has similar negative effects on the reproductive health and outcomes of ARTs (10–13). In contrast, there have been some studies showing that there is no measurable effect of recent or ongoing smoking on ARTs outcomes (4,14–16).

ARTs have physical, psychological, social, and economic effects on couples. Therefore, taking measures to increase the success of ARTs is an important issue concerning the entire healthcare team. The aim of the present study was to determine the effects of cotinine concentrations in the follicular fluid (FF) or seminal plasma (SP) of couples on the success rate of ARTs (pregnancy rate), since they were thought to inhibit the success of ARTs. Determination of cotinine concentrations in FF and SP due to cigarette smoking and/or exposure to secondhand smoke is important for counseling infertile couples to increase the chance of success with ARTs.

* Correspondence: FTerzioglu@istinye.edu.tr

We evaluated the cotinine concentrations in FF and SP in this study because cotinine is a reliable indicator to determine exposure to cigarette smoke. The half-life of nicotine is 2 to 3 h. Therefore, when a person is exposed to smoke, nicotine concentrations in biological fluids are not constant. However, cotinine has a longer half-life (17 h) than nicotine does. Because of its longer half-life, cotinine is eliminated from the body in a longer time compared with nicotine (17).

2. Materials and methods

2.1. Study design and sample

This study was designed as a case control study of 217 couples undergoing ARTs at an in vitro fertilization unit of a state hospital in Ankara, Turkey, with the objective of determining the effects of cotinine concentrations in the FF and SP of couples on the pregnancy outcome of ARTs.

Couples who started ARTs, were not diagnosed with any psychological disorder, were smokers at that time (regularly or occasionally), had never smoked (but may have been exposed to passive cigarette smoke), or were former smokers, and agreed to enroll in the study were included. Among these couples, there were nonsmokers (66 women and 40 men), passive smokers (106 women and 54 men), and active smokers (45 women and 123 men).

2.2. Ethical considerations

Ethical approval to conduct the study was given by the university ethics committee and the study hospital's ethics committee. Couples were informed about the study and asked to sign the informed consent document before the study.

2.3. Data collection

The study data were collected in two steps. In the first step, a semistructured data collection form was given to couples that began ARTs. The data collection form was filled out by the researchers in a face-to-face interview. In the second step, SP and FF samples were collected from the couples that were undergoing ARTs. The obtained samples were transferred to Gazi University Toxicology Department in accordance with cold chain principles to be analyzed for cotinine concentrations. The cotinine concentrations in the FF and SP samples were measured using the enzyme-linked immunosorbent assay (ELISA).

2.4. Preliminary study

A preliminary study was conducted with 20 couples to analyze whether or not the questionnaire could be understood and was usable. As a result of the preliminary study, questions that were not understandable were revised by the researchers and the questionnaire was put into its final format.

Furthermore, cotinine analysis was performed 10 times on the FF of 3 women and on the SP of 3 men who smoked one pack a day, smoked half a pack a day, and were nonsmokers using the immunoassay method.

2.5. Evaluation of smoking status

Couples included in the study were classified into three groups according to their smoking habits (18,19):

1) Nonsmokers, which means that both the husband and the wife are nonsmokers or former smokers,

2) Passive smokers, which means that the wife is a nonsmoker and the husband is a smoker, and

3) Smokers, which means that the wife currently smokes regularly or occasionally and the husband may or may not smoke.

2.6. Cotinine analysis

The half-life of cotinine is about 17 h while that of nicotine is about 2 h. Because of the longer half-life of cotinine and constant cotinine concentrations in biological fluids, it is the preferred measure to estimate nicotine exposure from tobacco (17,20).

All FF and SP samples were stored at −80 °C until the day of evaluation; cotinine concentrations were measured using a cotinine ELISA kit. Patients were grouped based on the levels of follicular and seminal fluid cotinine into three groups (15):

1) Cotinine levels for nonsmokers, ≤20 ng/mL,

2) Cotinine levels for passive smokers, >20 ng/mL but <50 ng/mL, and

3) Cotinine levels for smokers, ≥50 ng/mL.

2.7. Statistical analysis

The statistical analysis was performed using SPSS 19.0. Data were analyzed with percentages, means and standard deviations, tests of significance of the difference between the groups, Pearson chi-square tests, Fisher chi-square tests, one-way analysis of variance tests, t test for independent samples, and correspondence analysis.

3. Results

As shown in Table 1, the cotinine concentrations in the FF and SP of nonsmokers were significantly lower than those of the other groups ($P < 0.05$). The cotinine concentrations in the FF and SP of passive smokers were higher than those of nonsmokers, but the difference was not significant (Table 1, $P > 0.05$). The difference between the cotinine concentrations detected in the FF of women with positive pregnancy test results and women with negative pregnancy test results was statistically insignificant (Table 2, $P > 0.05$).

Correspondence analysis was performed for the characteristics that affect pregnancy status, the results of which showed that women aged 36 and over, having their second or more ART attempt had lower chances of pregnancy compared to the other groups. Women who never smoked, never had any women's disease, and who were underweight or normal in terms of body mass

Table 1. Comparison of cotinine concentrations in FF and SP according to smoking status and sex.

Sex	Smoking status[a]	n (217)	Cotinine concentrations (ng/mL, mean ± SD)	P
Female	Nonsmoker	66	3.66 ± 14.23	0.001
	Smoker	45	53.91 ± 44.45	
	Passive smoker	106	10.48 ± 31.55	
Male[b]	Nonsmoker	29	7.33 ± 22.62	0.001
	Smoker	99	67.90 ± 37.15	
	Passive smoker	38	8.01 ± 21.12	

[a]Smoking status was determined based on the number of cigarettes smoked.
[b]Sperm samples were collected from 166 infertile men.

Table 2. Comparison of cotinine concentrations in FF according to pregnancy results.

Pregnancy result	n (217)	Cotinine concentration (ng/mL, mean ± SD)	P
Positive	70	19.42 ± 39.154	0.573
Negative	147	16.46 ± 34.718	

index had greater chances of pregnancy than the other groups, while women who were smokers, had any type of women's disease, and were overweight had lower chances of pregnancy than the other groups (Figure).

The rate of clinical pregnancy was 24.1% for nonsmoker women and 25.6% for passive smoker or smoker women (Table 3, $P > 0.05$). It was also determined that while the rate of clinical pregnancy was 24.7% for the women whose husbands were nonsmokers, the rate of clinical pregnancy was 28.7% for the women whose husbands were passive smokers or smokers (Table 4, $P > 0.05$).

4. Discussion

There is strong evidence that smoking negatively affects almost all aspects of reproduction including follicular development/ovulation, oocyte retrieval and egg transport in the fallopian tube, fertilization, and embryo development (21–23). The substances in cigarette smoke have toxic effects on the ovaries and the testes. Benzopyrene, cadmium, and cotinine, the metabolite of nicotine, reach the ovarian follicles of smoker or passive smoker women and reduce the fertilization ability of the oocyte (24).

In the literature, there are many studies that have determined cotinine concentrations in FF to measure exposure to smoke. Even though the findings obtained from these studies differ, these studies found that cotinine concentrations in FF were higher for smokers than for the other groups (passive smokers and nonsmokers) (18,19,24–28). In our study, we found similar results as shown in Table 1. Our results demonstrated that, among smokers, cotinine concentrations in FF (53.91 ± 44.45 ng/mL) were significantly higher than those of the other groups, at 10.48 ± 31.55 ng/mL and 3.66 ± 14.23 ng/mL for passive smoker women and nonsmoker women, respectively ($P < 0.05$).

Some studies indicate that cotinine is detectable in the FF of women who declared themselves as nonsmokers and a large portion of these women were exposed to cigarette smoke (29–32). In our study, cotinine concentrations in the FF of 66 nonsmoker women were 3.66 ± 14.23 ng/mL (Table 1). This result shows that these women were exposed to cigarette smoke.

There is a lack of research about the effects of paternal smoking on ARTs because problems related to reproduction (e.g., abortion) have traditionally been associated with women. The results obtained from studies on cotinine concentrations in SP differ. Shen et al. (33) and Sergerie et al. (29) found that smokers had substantially higher cotinine concentrations in their SP. However, Wong et al. found no correlation in terms of seminal cotinine concentrations in smoker and nonsmoker men (34). We found that among smokers the mean cotinine concentrations in the SP (67.90 ± 37.15 ng/mL) were significantly higher than those of the other groups, at 8.01 ± 21.12 ng/mL for passive smoker men and 7.33 ± 22.62 ng/mL for nonsmoker men (Table 1, $P < 0.05$).

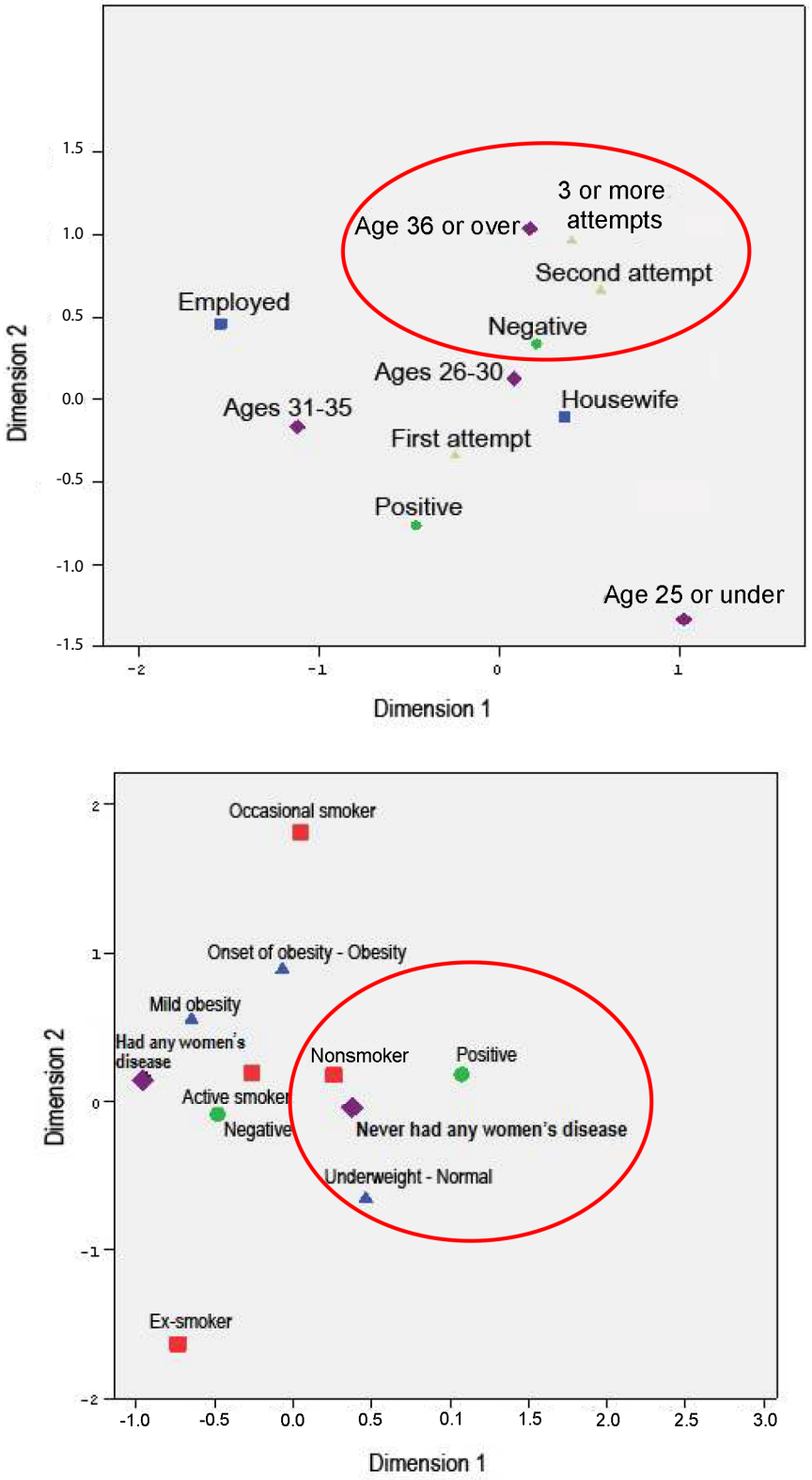

Figure. Review of the independent variables that have an influence on pregnancy in women (correspondence analysis).

Table 3. Comparison of pregnancy results according to cotinine concentrations in FF.

Pregnancy status	Nonsmoker[a] (n = 174)	Passive smoker or smoker[b] (n = 43)	P
No pregnancy[c]	132 (75.9%)	32 (74.4%)	0.085
Clinical pregnancy	42 (24.1%)	11 (25.6%)	

[a]The assigned cotinine concentration for nonsmokers was ≤20 ng/mL.
[b]The assigned cotinine concentration for smokers was >20 ng/mL
[c]Four nonsmoker women and 4 passive smoker or smoker women had chemical pregnancies.

Table 4. Comparison of outcomes of pregnancy in wives according to cotinine concentrations in SP.

Pregnancy status of wife	Nonsmoker[a] (n = 77)	Passive smoker or smoker[b] (n = 87)	P
No pregnancy[c]	58 (75.3%)	62 (71.3%)	0.470
Clinical pregnancy	19 (24.7%)	25 (28.7%)	

[a]The assigned cotinine concentration for nonsmokers was ≤20 ng/mL.
[b]The assigned cotinine concentration for smokers was >20 ng/mL.
[c]Two women whose husbands were nonsmokers and 5 women whose husbands were passive smokers or smokers had chemical pregnancies.

The results of studies on the effects of female cigarette smoking on the outcomes of ARTs are complex. In a study conducted with 45 women by Rosevear et al., 116 eggs were collected from 32 women with no detectable FF cotinine and 84 eggs could be fertilized (72%); at the same time, 45 eggs were collected from 13 women with detectable FF cotinine and 20 eggs could be fertilized (44%) ($P < 0.01$). They also found that the median fertilization rates were 57% for women whose cotinine concentrations were high and 75% for women whose cotinine levels were low (30). Al-Saleh et al. found that DNA adducts in the FF of women who could not become pregnant were higher than those of pregnant women ($P < 0.05$). They also determined that there was a significant relationship between cotinine concentrations in FF and the level of DNA adducts ($P < 0.05$) (35). In another study conducted by Al-Saleh et al., the cotinine concentration in the FF was 1.72 ± 12.18 for pregnant women and 2.68 ± 18.95 for women who could not become pregnant ($P > 0.05$) (31). Fuentes et al. determined that 7 of 94 embryos transferred to smoker women, whose FF cotinine concentrations were >10 ng/mL, were successfully implanted (7.4%) and 56 of 378 embryos transferred to nonsmoker women, whose FF cotinine concentrations were not detectable, were successfully implanted (14.81%) ($P > 0.05$). The authors also found that 10 of 33 smoker women (30%) and 47 of 133 nonsmoker women achieved a positive pregnancy (35.3%) ($P > 0.05$) (28). However, some studies reported that smoking has no effect on pregnancy rates (4,6,14,15,34,36–38). In our study, we determined that the difference in FF cotinine concentrations between women with positive pregnancy test results and women with negative pregnancy test results was statistically insignificant (Table 2, $P > 0.05$). It was also found that there was an insignificant difference between the percentage of clinical pregnancy in nonsmoker and the percentage of clinical pregnancy in passive smoker or smoker women (Table 3, $P > 0.05$).

Available data about the effect of men's smoking on ART outcomes are not clear. In a study conducted by Pacifici et al., sperm specimens from 44 smokers and 50 nonsmokers were analyzed. They found no significant correlation between sperm concentrations and SP cotinine concentrations (39). In a comparative study conducted by Hassan et al., it was found that there was a negative correlation between SP cotinine concentrations and sperm motility (40). Hugges et al. conducted a prospective study in which maternal smoking was monitored for 316 couples that underwent ART. They reported that there was no correlation between paternal smoking and reduction in conception rate (37). In another study, conducted by Gandini et al., sperm specimens from 10 healthy men aged 28 to 35 were analyzed. They suggested that nicotine and cotinine are not responsible for the harmful effects of cigarette smoke on sperm kinetic parameters reported in the literature (32). In our study, we found that the difference in percentage of clinical pregnancy between

women whose husbands were nonsmokers and women whose husbands were smokers or passive smokers was statistically insignificant (Table 4, $P > 0.05$).

In conclusion, despite the differences between the studies, there is convincing evidence that smoking has a negative effect on ART outcomes. Although we found that the percentage of clinical pregnancy in passive smoker or smoker women was higher than that in nonsmoker women, not smoking or quitting smoking before starting treatment is extremely important for couples to achieve successful outcomes. Because smoking is a modifiable and controllable risk factor driven by individual desire, quitting smoking must be a prerequisite for couples that will undergo ARTs. A change to a healthy lifestyle may reduce the need for invasive therapy handled with high-cost techniques (ARTs, ETs, etc.) and increase the effectiveness of treatment.

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