CLINICAL ASSISTED REPRODUCTION

The Effect of Smoking on Oocyte Quality and Hormonal Parameters of Patients Undergoing In Vitro Fertilization–Embryo Transfer

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Purpose: *The aim of the present study was to investigate the influence of smoking on different parameters such as oocyte count, embryo score, and basal hormone values within the scope of in vitro fertilization–embryo transfer (IVF-ET).*

Methods: *Eight hundred thirty-four women undergoing IVF-ET treatment were classified as smokers or nonsmokers on the basis of questionnaires. Additionally, we divided them into three groups according to their stimulation protocol—"combined stimulation"[I; clomiphene citrate plus human menopausal gonadotropin (hMG)], "ultrashort" [II; gonadotropin releasing hormone agonist (GnRHa) plus hMG or follicle-stimulating hormone (FSH)], and "long downregulation protocol" (III)—and further classified again as smokers or nonsmokers within the groups.*

Results: *In general, smoking patients were significantly ($P = 0.0195$) younger than nonsmokers and showed a significantly ($P = 0.0379$) lower embryo score and a tendency ($P = 0.0931$) to produce fewer oocytes. There was no significant difference concerning the number of normally or pathologically fertilized and transfered oocytes and embryos suitable for cryopreservation. Women who smoked had significantly ($P = 0.0112$) higher basal 17-β-estradiol (E_2), luteinizing hormone (LH) ($P = 0.0001$), and dehydroepiandrosteronesulfate (DHEAS) ($P = 0.0039$) levels, but their basal human prolactin (HPRL) levels were significantly ($P = 0.0033$) lower than those of nonsmokers. According to the stimulation protocol used, we found the following results. Smoking patients in group I showed a significantly ($P = 0.023$) lower embryo score and produced fewer oocytes ($P = 0.0113$), with fewer of them being fertilized ($P = 0.0072$) and transferred ($P = 0.0067$). Women who smoked had significantly ($P = 0.0002$) higher basal LH levels, but their HPRL levels were significantly ($P = 0.031$) lower than those of nonsmokers. Furthermore, they had a thinner endometrium on the day of embryo transfer ($P = 0.0366$). In group II we measured significantly elevated basal E_2 levels ($P = 0.0089$) and higher LH values ($P = 0.0092$) in smokers. Group III showed a trend ($P = 0.0565$) toward lower HPRL values in smokers.*

Conclusions: *Although the fertilization rate of oocytes and the pregnancy rate were not significantly different between smokers and nonsmokers, we found significantly alterated hormonal parameters and negatively influenced oocyte parameters, particularly after clomiphene stimulation. So we might consider using only GnRHa protocols for smoking patients. Additionally, we advise our patients to stop smoking before an IVF-ET treatment because of the complex effects of smoking on the reproductive and hormonal system.*

KEY WORDS: embryo score; estradiol; in vitro fertilization; luteinizing hormone; smoking.

INTRODUCTION

The association between smoking and female infertility has been discussed in many studies. Jick *et al.* (1) observed a shorter reproductive phase, an earlier onset

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of menopause, and a reduction in fertility among smokers in comparison with nonsmokers. Baird and Wilcox (2) and Howe *et al.* (3) confirmed these results. Feichtinger *et al.* showed, in their meta-analysis (4), a significantly lower pregnancy rate among smokers. Remmer and Schindler (5) reported a higher spontaneous abortion rate and premature birth rate and, in addition, an influence on fetal development.

Under controlled conditions in vitro fertilization–embryo transfer (IVT-ET) offers the possibility to investigate the influence of nicotine and other tobacco contents on biological factors. The purpose of this study was to accurately analyze oocyte quality, hormone parameters of the pituitary system, stimulation of ovaries, endometrium, oocytes, fertilization rate, and implantation.

METHODS

In this retrospective study the IVF database of our institute was used to obtain information on the smoking status of women undergoing IVF-ET. Beginning hormonal stimulation in the scope of IVF-ET, the patients were classified as nonsmokers or smokers, the latter according to their habits: light (1–9 cigarettes per day), medium (10–20 cigarettes per day), and heavy (more than 20 cigarettes per day) smokers. The height and weight of the patients were recorded and the following data were obtained in every group: follicle-stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfate (DHEAS), human prolactin (HPRL), and 17-β-estradiol (E_2) on the second or third day of the cycle.

According to the stimulation protocols, we obtained three groups. Group I (332 patients) received combined stimulation with clomiphene citrate and human menopausal gonadotropin (hMG) (8). The patients in group II (433 patients) were treated with an "ultrashort flare-up protocol" (6) with the gonadotropin releasing hormone agonist (GnRHa) Buserelin and pure follicle-stimulating hormone (FSH). Group III (73 patients) was stimulated with a long downregulation protocol (GnRHa-FSH) (7).

The quality of the embryo, the so-called "embryo score," was assessed prior to ET according to the method of Steer *et al.* (9): Grade 4, equal-sized symmetrical blastomeres; Grade 3, uneven blastomeres with <10% fragmentation; Grade 2, 10–50% blastomeric fragmentation; and Grade 1, >50% blastomeric fragmentation or pronucleate single-cell embryos. The morphological grade of the embryo was then multiplied by the number of blastomeres.

Additional variables were the number of oocytes recovered during follicle aspiration, pathological fertilization (three or more pronuclei, no further development), and those oocytes which were used for embryo transfer and those used for freezing (Grade 4).

Statistical analysis was performed using a standardized computer program (Stat View; Abacus Concepts, USA). The following tests were used: chi-square test, Student's *t* test, and analysis of variance (ANOVA).

RESULTS

Eight hundred thirty-four women undergoing IVF-ET were classified into nonsmokers (75.97%) and smokers (24.03%) on the basis of questionnaires. Smoking patients (33.27 ± 4.93 years) were significantly ($P = 0.0195$) younger than nonsmoking ones (34.19 ± 5.33). Height and weight were not significantly different between nonsmokers and smokers. The reasons for sterility and indication for IVF-ET were equally distributed in both groups.

Analysis of the hormone parameters showed significant differences between smokers and nonsmokers (Fig. 1). The mean baseline LH levels (days 2–3) were significantly ($P = 0.0001$) higher in smokers (5.26 ± 3.35 mU/ml) versus nonsmokers (3.89 ± 2.35 mU/ml). A significantly ($P = 0.0033$) lower basal HPRL was measured in smokers (15.96 ± 7.08 ng/ml) versus nonsmokers (19.23 ± 10.69 ng/ml). A significant ($P = 0.0039$) difference was found in the mean DHEAS levels in women who smoked (1.38 ± 0.71 μg/ml) versus in those who did not (1.13 ± 0.72 μg/ml). Basal E_2 was significantly ($P = 0.0112$) higher in smokers (39.11 ± 43.37 pg/ml) than in nonsmokers (30.55 ± 18.85 pg/ml). However, mean basal FSH levels showed no statistical difference (9.56 mU/ml ± 4.42 in smokers and 9.36 mU/ml ± 5.95 in nonsmokers).

Among smokers there was a slight trend toward a lower oocyte count at oocyte recovery (6.44 ± 3.68 in smokers versus 6.99 ± 4.09 in nonsmokers). In terms of the number of pathologically fertilized oocytes as well as those which were suitable for cryopreservation, we found no significant differences between the two groups (Table I). The quality of fertilized oocytes, i.e., the embryo score, was significantly ($P = 0.0379$) lower in smokers (38.89 ± 21.56) than in nonsmokers (43.08 ± 24.24).

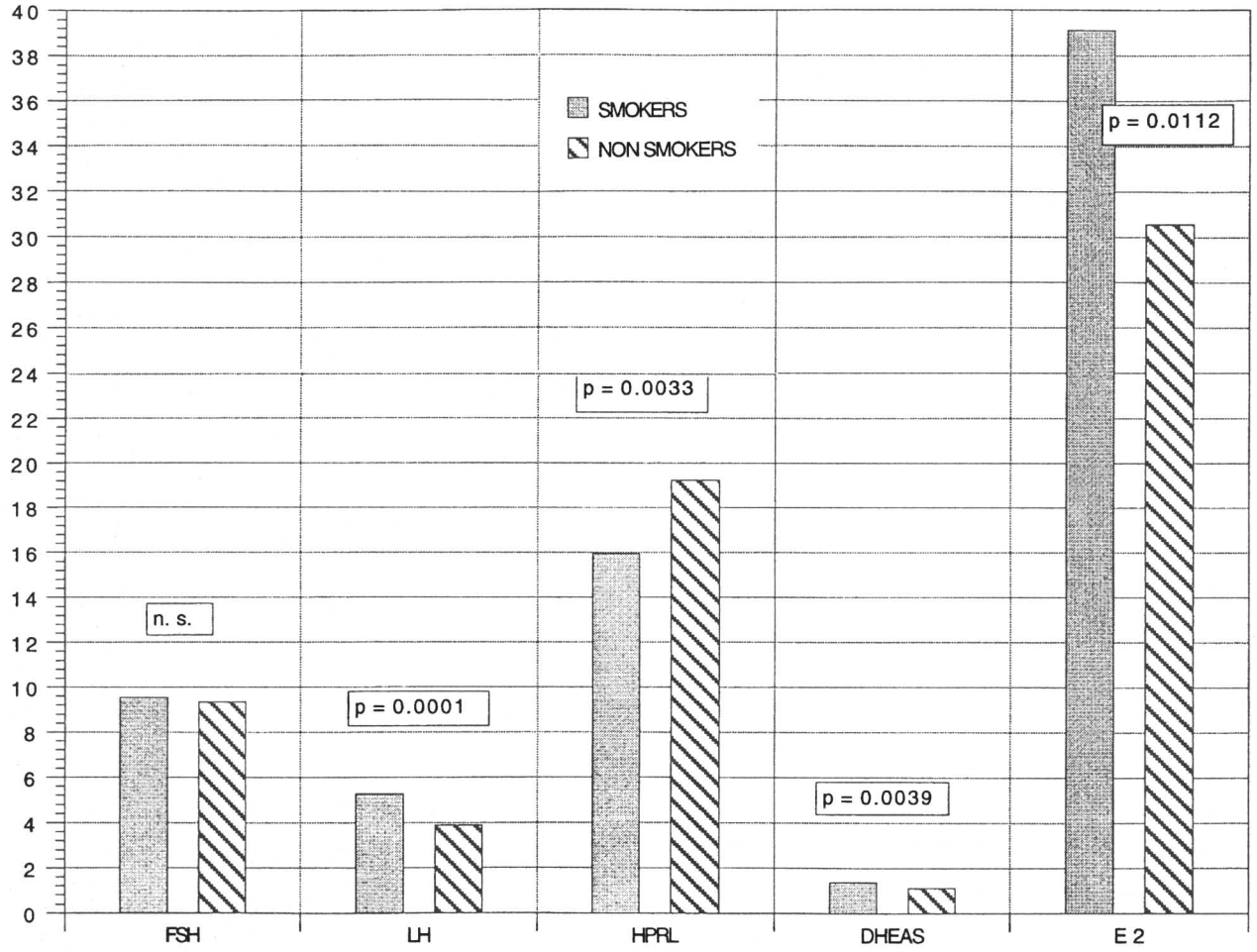

Fig. 1. The hormonal parameters FSH, LH, HPRL, DHEAS, and E_2 in a direct comparison between smokers and nonsmokers (all patients).

The cancellation rate was 17.6% among patients who smoked and 14.5% among nonsmokers, for various reasons (e.g., lack of follicle growth, a lower oocyte count, i.e., "low response"). There was a slightly higher pregnancy rate in nonsmokers (30.55%) versus smokers (24.30%; not significant), but this has been reported for the same patient material elsewhere (4). The abortion and ectopic pregnancy rates were not different between the two groups.

According to the stimulation protocol used, we obtained the following results. Group I (Table II), treated with clomiphene citrate and hMG, consisted

Table I. Variance Analysis of Different Variables from the IVF-ET Program in Relation to Smoking Status

	Nonsmokers	Smokers	*P*
No. of oocytes	6.99 ± 4.09	6.44 ± 3.68	0.0931
No. of fertilized oocytes	4.26 ± 2.83	3.92 ± 2.58	n.s.
No. of oocytes reserved for cryopreservation	1.09 ± 2.23	0.96 ± 1.97	n.s.
No. of pathological oocytes	0.17 ± 0.45	0.19 ± 0.57	n.s.
No. of embryos transferred	2.46 ± 0.89	2.33 ± 0.92	n.s.
Embryo score	43.08 ± 24.24	38.89 ± 21.56	0.0379
Thickness of endometrium	12.69 ± 3.62	12.26 ± 3.63	n.s.

Table II. Results According to the Stimulation Protocol

	Group I: Clomiphene citrate and hMG stimulation			Group II: Ultrashort flareup protocol, GnRH, and FSH			Group III: Long down regulation protocol, GnRH, and FSH		
	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*	Nonsmokers	Smokers	*P*
Embryo score	**43.53 ± 23.27**	**36.78 ± 22.20**	**0.023**	43.29 ± 25.04	40.69 ± 20.48	n.s	40.62 ± 24.53	40.42 ± 25.17	n.s
No. of oocytes	**6.14 ± 3.36**	**5.14 ± 2.53**	**0.0113**	7.49 ± 4.40	7.51 ± 3.94	n.s.	8.02 ± 4,59	7.58 ± 5.6	n.s
No. of fertilized oocytes	**4.18 ± 2.65**	**3.33 ± 2.02**	**0.0072**	4.29 ± 2.95	4.42 ± 2.76	n.s.	4,46 ± 3.01	4.33 ± 3.92	n.s
No. of cryopreserved oocytes	1.11 ± 2.07	0.77 ± 1.47	n.s	1.06 ± 2.34	1.17 ± 2.37	n.s	1.20 ± 2.35	0.08 ± 0.29	n.s
No. of pathological oocytes	0.18 ± 0.46	0.18 ± 0.66	n.s	0.17 ± 0.44	0.20 ± 0.50	n.s	0.16 ± 0.45	0.67 ± 1.61	n.s
No. of embryos transferred	**2.39 ± 0.84**	**2.10 ± 0.88**	**0.0067**	2.50 ± 0.94	2.56 ± 0.90	n.s	2.54 ± 0.91	2.17 ± 0.94	n.s
Thickness of endometrium	**12.83 ± 3.43**	**11.90 ± 3.95**	**0.0366**	12.46 ± 3.95	12.65 ± 3.46	n.s	**13.18 ± 2.43**	**11.92 ± 2.23**	**0.099**
FSH	9.17 ± 6.67	9.70 ± 4.15	n.s.	9.69 ± 5.46	9.57 ± 4.83	n.s.	7.56 ± 4.26	6.57 ± 0.87	n.s
LH	**4.00 ± 2.45**	**5.59 ± 2.79**	**0.0002**	**3.81 ± 2.29**	**4.99 ± 3.93**	**0.0092**	3.95 ± 2.30	3.27 ± 1.78	n.s
E_2	33.10 ± 20.35	34.43 ± 14.55	n.s.	**28.8 ± 17.74**	**44.88 ± 63.28**	**0.0089**	29.56 ± 17.45	37.00 ± 13.75	n.s
HPRL	**21.29 ± 11.31**	**16.32 ± 6.67**	**0.0031**	17.59 ± 9.99	16.06 ± 7.61	n.s	**19.34 ± 9.52**	**9.23 ± 2.94**	**0.056**
DHEAS	3.09 ± 16.66	1.44 ± 0.79	n.s	25.08 ± 250.79	11.8 ± 63.63	n.s	1.48 ± 1.55	1.09 ± 0.59	n.s

of 245 nonsmokers and 87 smokers. Analysis of the hormone parameters showed significant differences between smokers and nonsmokers. The mean baseline LH level (days 2–3) was significantly ($P = 0.0002$) higher in smokers (5.59 ± 2.79 mU/ml) versus nonsmokers (4.00 ± 2.45 mU/ml). A significantly ($P = 0.0031$) lower basal HPRL was measured in smokers (16.32 ± 6.67 ng/ml) versus nonsmokers (21.29 ± 11.31 ng/ml). However, mean basal FSH levels, DHEAS, and E_2 showed no statistical difference between smokers and nonsmokers.

Among smokers a significantly ($P = 0.0113$) lower number of oocytes was retrieved (5.14 ± 2.53 versus 6.14 ± 3.36), a significantly ($P = 0.0072$) lower number of oocytes was fertilized (3.33 ± 2.02 versus 4.18 ± 2.65), and significantly ($P = 0.0067$) fewer of them were suitable for embryo transfer (2.10 ± 0.88 versus 2.39 ± 0.84). The quality of fertilized oocytes, i.e., the embryo score was also significantly ($P = 0.023$) lower in smokers (36.78 ± 22.20) than in nonsmokers (43.53 ± 23.27). In terms of the number of pathologically fertilized oocytes as well as those which were suitable for cryopreservation, we found no significant difference between nonsmokers and smokers. The cancellation rate because of different reasons was higher among patients who smoked (20.91%) than among nonsmokers (13.43%; $P = 0.0922$). Furthermore, the thickness of the endometrium on the day of ET was significantly ($P = 0.0366$) higher in nonsmokers. We found no significant difference in the pregnancy rate: 35.10% of nonsmokers and 29.89% of smokers became pregnant (Table III).

Group II (Table II), treated with an "ultrashort protocol," included 328 nonsmokers and 105 smokers. Among the hormonal parameters assessed, the basal E_2 levels (44.88 ± 63.28 versus 28.8 ± 17.74 pg/ml) as well as the LH values (4.99 ± 3.93 versus 3.81 ± 2.29 mU/ml) were significantly elevated in smoking patients ($P = 0.0089$ for E_2 versus $P = 0.0092$ for LH levels). The levels of HPRL, FSH, and DHEAS were equally distributed in both groups.

Other recorded parameters (number of recovered, normal and pathologically fertilized, cryopreserved, and transferred oocytes, embryo score, and endometrium thickness) showed no significant differences in comparisons between smokers and nonsmokers. Finally, we did not find a significantly different pregnancy rate (26.36% in smokers versus 25.42% in nonsmokers) or cancellation rate (15.45% in smokers versus 16.20% in nonsmokers) (Table III).

Group III (Table II) consisted of 61 nonsmokers and 12 smokers stimulated with the long protocol. While FSH, LH, E_2, and DHEAS levels were not significantly altered in smokers, HPRL showed a trend ($P = 0.0535$) toward lower values (9.23 ± 2.94 in smokers versus 19.34 ± 9.52 ng/ml in nonsmokers). Sixteen and sixty-seven hundredths percent of smoking patients and 31.15% of nonsmokers became pregnant ($P = 0.5066$). While the cancellation rate was 8.96% in nonsmokers in this stimulation group, no cycle had to be cancelled in smokers ($P = 0.289$) (Table III).

There were no differences whatsoever when patients were again subdivided according to the number of cigarettes smoked (light, medium, and heavy smokers), but in this calculation the groups had become quite small.

Table III. Comparison of Pregnancy and Cancellation Rates Between Nonsmokers and Smokers

Stimulation protocol	Nonsmokers	Smokers	*P*
Pregnancy rate (%)			
Group I	35.10	29.89	0.4520
Group II	25.42	26.36	0.9709
Group III	31.15	16.67	0.5066
Cancellation rate (%)			
Group I	13.43	20.91	0.0922
Group II	16.20	15.45	0.9709
Group III	8.96	0	0.2890

DISCUSSION

Smoking is emerging as an increasingly important factor in IVF-ET. This is because smokers constitute a considerable proportion of patients who undergo IVF-ET and the consumption of tobacco is steadily increasing, especially among younger women. This epidemiologic trend was also observed in our study—smokers were significantly younger than nonsmoking women.

Smoking influences the reproductive system and therefore, apperently, IVF-ET treatment and its hormonal parameters. In our overall results the E_2 levels determined on the second or third day of the menstrual cycle were significantly higher. One explanation for this phenomenon is offered by Bodis *et al.* (10), who describe the effect of nicotine on human follicular cells in vitro. The authors report that nicotine caused a dose-dependent increase in E_2 secretion. Elevated E_2 levels may indicate an impaired ovarian response and result in a higher cancellation rate and a lower oocyte yield (11). A further interesting observation was that prolactin levels were generally significantly lower among

smokers (especially in Group I, and Group III showed a trend toward decreased levels). This was confirmed by Berta *et al.* (12), who postulated that nicotine causes a central release of dopamine and thus inhibits the secretion of prolactin. Physiologically, the enhanced prolactin level after parturition has a modulating effect on the FSH/LH mechanism, which prevents a subsequent early pregnancy (13). Therefore, a lower prolactin level should theoretically have a positive effect on fertility rates. However, it was reported in the literature (14) that fertility may also be influenced negatively when prolactin levels are too low. We therefore believe that the influence of HPRL on IVF parameters must be studied further.

Furthermore, we found altered results concerning oocyte number and quality depending on smoking status and the stimulation protocol used. In Group I a lower oocyte count and a poor oocyte quality, manifested by a lower embryo score, were recorded. Premature atresia or damaged oocytes may be caused by several harmful substances (rhodanite, cotinine, cadmium) that accumulate in the follicular fluid of smokers.

Trapp *et al.* (15) proved the existence of rhodanite, a substance found in tobacco, in the follicular fluid. This was an indication of the number of cigarettes consumed. Zenzes *et al.* (16) reported a significant, dose-dependent increase in cadmium levels in the follicular fluid of smokers. They suspect that the accumulation of heavy metal in tobacco impairs the normal course of meiotic cell division by inhibiting the spindle apparatus function of maturing oocytes and causes chromosomal abnormalities and a lower number of oocytes that reach full maturity. In a subsequent study Zenzes *et al.* (17) observed a correlation between the number of cigarettes smoked per day and elevated cotinine levels in the follicular fluid and serum of smoking patients.

We also attempted to recalculate our data according to the number of cigarettes smoked (light, medium, or heavy), but no statistical differences could be shown among these groups. This however, could be due partly, to the fact that the calculated numbers became quite small in some groups. The influence of smoking on pregnancy rates has been controversially discussed in various studies. Some authors [Elenbogen *et al.* (18), Rosevear *et al.* (19), and Harrison *et al.* (20)] observed a lower number of pregnancies among women who smoked, while Trapp *et al.* (15), Hughes *et al.* (21), and Sterzik *et al.* (22) observed no significant difference between the two groups of patients.

A meta-analysis recently published by Feichtinger et al. (4), which reports the authors' own data as well as the results of seven other important publications, mentions significant differences between the two groups. The authors performed a statistical meta-analysis of 2314 first cycles of IVF-ET treatment, showing that the pregnancy rate among nonsmokers (21%) was significantly higher than that among smokers (14%).

In conclusion the results of the present study did not demonstrate significantly impaired pregnancy or cancellation rates in smoking patients. However, we found significantly altered hormonal parameters, a negatively influenced oocyte count, fertilization rate, and embryo score, particularly in the group stimulated with a combined clomiphene citrate/hMG protocol. Therefore we might consider using only GnRHa protocols for smoking patients.

Additionally we advise our patients to abandon smoking before an IVF-ET treatment, in view of earlier results (4,18,19,20,23) as well the complex effects of smoking on hormonal parameters and oocyte number and quality.

REFERENCES

1. Jick H, Porter J, Morrison AS: Relation between smoking and age of natural menopause. Lancet 1977;1:1354–1355
2. Baird DD, Wilcox AJ: Cigarette smoking associated with delayed conception. JAMA 1985;253:2979–2983
3. Howe G, Westhoff C, Vessey M, Yeates D: Effects of age, cigarette smoking, and other factors on fertility: Findings in a large prospective study. Br Med J 1985;290:1697–1700
4. Feichtinger W, Papalambrou K, Poehl M, Krischker U, Neumann K: Smoking and in vitro fertilization: A meta-analysis. J Assist Reprod Genet 1997;14:596–599
5. Remmer H, Schindler AE: Prävention in Gynäkologie und Geburtshilfe. Terramed Überlingen 1986;XX:243–265
6. Macnamee CM, Howles CM, Edwards RG, Taylor PJ, Elder KT: Short luteinizing hormone-releasing agonist treatment: Prospective trial of a novel ovarian stimulation regimen for in-vitro fertilization. Fertil Steril 1989;19:89–92
7. Tan SL, Kingsland C, Campell S, Mills C, Bradfield J, Alexander N, Yovich J, Jacob HS: The long protocol of administration of gonadotropin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for in-vitro fertilization. Fertil Steril 1992;57:810–814
8. Kemeter P, Feichtinger W: Experience with a new fixed-stimulation protocol without hormone derterminations for programmed oozyte retrieval for in-vitro fertilization. Hum Reprod 1989;4:53–58
9. Steer CV, Mills CL, Tan SL: The cumulative embryo score: A predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. Hum Reprod 1992;117–119
10. Bodis J, Hanf V, Torok A, Tinneberg H-R: Smoking and corpus luteum function. Fertil Steril 1996;4:810–814

11. Evers J, Slaats P, Land J, Dumoulin J, Dunselman G: Elevated levels of basal estradiol-17β predict poor response in patients with normal basal levels of follicle stimulating hormone undergoing in vitro fertilization. Fertil Steril 1998;69:1010–1014
12. Berta L, Fortunati N, Gennari P, Appendino M, Casella A, Frairia R: Influence of cigarette smoking on pituitary and sex hormone balance in healthy premenopausal women. Fertil Steril 1991;56:788–789
13. Leidenberg FA: Klinische Endokrinologie für Frauenärzte. Berlin, Heidelberg, Springer Verlag, 1992, pp 57–58
14. Huber J: Endokrine Gynäkologie. Zürich, Wien, Verlag Maudrich, 1998, p 23
15. Trapp M, Kemeter P, Feichtinger W: Smoking and in-vitro fertilization. Hum Reprod 1986;6:357–358
16. Zenzes MT, Krishnan S, Krishnan B, Zhang H, Casper RF: Cadmium accumulation in follicular fluid of women in in-vitro fertilization-embryo transfer is higher in smokers. Fertil Steril 1995;64:599–603
17. Zenzes MT, Reed TE, Wang P, Klein J: Cotinine, a maior metabolite of nicotine, is detectable in follicular fluid of women in in-vitro fertilization therapy. Fertil Steril 1996;66:614–619
18. Elenbogen A, Lipitz S, Mashiach S, Dor J, Levran D, Ben-Rafael Z: The effect of smoking on the outcome of in-vitro fertilization-embryo transfer. Hum Reprod 1991;6:242–244
19. Rosevar SK, Holt PW, Lee TD, Ford WC, Wardle PG, Hull MG: Smoking and decreased fertilization rates in-vitro. Lancet 1992;340:1195–1196
20. Harrison K, Breen T, Henessey J: The effect of patient smoking on the outcome of IVF and GIFT treatment. Aust NZ J Obstet Gynaecol 1990;30:340–343
21. Hughes E, Yeo J, Claman P, YoungLai E, Sagle M, Daya S, Collins J: Cigarette smoking and the outcome of in vitro fertilization: measurement of effect size and levels of action. Fertil Steril 1994;62:807–814
22. Sterzik K, Strehler E, De Santo M, Trumpp N, Abt M, Rosenbusch B: Influence of smoking on fertility in women attending an in-vitro fertilization program. Fertil Steril 1996;4:810–814
23. Pattinson HA, Taylor PJ, Pattinson MH: The effect of cigarette smoking on ovarian function and early pregnancy outcome of in-vitro fertilization treatment. Fertil Steril 1991;55: 780–783