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Letters to the Editor

The in-vitro effects of nicotine and cotinine on sperm motility

Dear Sir,

I read with interest the paper by Gandini *et al.* (1997) concerning the negative role of in-vitro nicotine and cotinine on sperm motility. We have recently undertaken a study of cigarette smoking on sperm parameters including motility. In the biochemically validated investigation we found no significant relationship between urinary excretion of nicotine metabolites (a measure of nicotine intake from cigarettes) and sperm motility. This is contrary to a similar study which found logged urinary cotinine (a major metabolite of nicotine) excretion to be negatively associated with the total number and the concentration of motile sperm (Vine *et al.*, 1996). These equivocal findings do little to resolve the hypothesis that smoking reduces sperm motility.

The second experiment conducted by Gandini *et al.* (1997) investigated the effects of in-toto aspirated cigarette smoke exposure on sperm cultures, and found a sharp reduction in all the sperm kinetic parameters. The authors demonstrated the effect was due to action on the moving apparatus, and excluded a cytotoxic action of the smoke on spermatozoa. However, they failed to put forward a possible mechanism. This experiment was similar to one undertaken by ourselves some while ago, to investigate the genotoxic effect of cigarette smoke. We exposed a suspension of lymphocytes to an in-toto solution of cigarette smoke produced by bubbling cigarette smoke through phosphate-buffered saline. The solution produced non-cytotoxic single-strand breaks to the lymphocyte DNA. The use of free radical scavengers showed these effects to be due to hydrogen peroxide, known to be in high concentrations in cigarette smoke (unpublished data).

This finding of the effects of aspirated cigarette smoke lends weight to the hypothesis that reactive oxygen species, generated from leukocyte contamination of semen, are responsible for decreased capacity of sperm movement (Aitken *et al.*, 1995), an effect which can be inhibited by antioxidants (Baker *et al.*, 1996).

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Dear Sir,

We thank Dr Cope (1998) for his interest in our article (Gandini *et al.*, 1997) with regard to in-vitro effects of nicotine and cotinine on sperm motility. He observes correctly that the controversial results on nicotine intake and sperm motility do little to resolve the hypothesis that smoking reduces sperm motility. Moreover, he suggests that the effects of cigarette smoke *in toto* on sperm motility are caused by reactive oxygen species generated from leukocyte contamination of semen and that these effects can be inhibited by antioxidants.

With regard to the negative role of nicotine and cotinine on sperm motility, we would like to underline that our study demonstrated that, *in vitro*, nicotine and cotinine added to a pool of spermatozoa separated by swim-up (at the levels found in the seminal plasma of smokers), do not affect sperm motility. A marked worsening of all sperm kinetic parameters was seen when we used doses of nicotine and cotinine 500 times more concentrated than the previous level.

In a previous study, we tested the seminal plasma of smokers and non-smokers for nicotine and cotinine and showed that there are high levels of these substances in smokers' seminal plasma for the same level present in blood. A comparison of the seminal parameters of smokers and non-smokers clearly indicated a significant reduction of the percentage of motile spermatozoa in smokers (Pacifici *et al.*, 1993), in agreement with Vine (1996). It would certainly interest us to see some of Dr Cope's own data.

With regard to the harmful effects of free radicals produced by leukocytes, we are firmly convinced that these products play a fundamental role, so much so that our group have proposed an antioxidant therapy to improve sperm kinetic characteristics (Lenzi *et al.*, 1993, l994). We have also investigated the effects of lipid peroxidation of the sperm membrane and the role of polyunsaturated fatty acids (Lenzi *et al.*, 1996). However, in the case of our experiment on the effects of cigarette smoke *in vitro*, the free radicals produced by leukocyte contamination do not seem to be responsible for the harmful effect seen on the sperm kinetics, in that our in-vitro model did not employ raw seminal fluid but a suspension of only motile sperm obtained by swim-up. Therefore, it seems more likely to us that other compounds, i.e. hydrocarbons, aldehydes, ketones, etc., found in the gaseous phase of the cigarette smoke, are responsible for the sperm motility damage.

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Theories of the pathophysiology of ovarian hyperstimulation syndrome should be based on the newest knowledge

Dear Sir,

With great interest have we read the paper by Elchalal and Schenker (1997). They give a summary of recent results in the field of ovarian physiology and propose a new theory of ovarian hyperstimulation (OHSS). On the basis of observed correlation between plasma cytokine activities and the severity of OHSS they suggest their involvement in the pathogenesis of OHSS. We agree that recent observations should be incorporated into the explanatory models of those syndromes, which are still poorly understood.

We believe that explanation of OHSS should stem from the physiological process itself. In this context, ovarian hyperstimulation is characterized as many synchronized follicular maturations and ovulations (quantitative changes), which induce some pathophysiological pathways that are responsible for the development of the symptoms of OHSS (qualitative changes).

There are some clinical similarities between pre-eclampsia and OHSS. Oedematous reaction, haemoconcentration, increase in heart rate and cardiac output, stimulation of the renin–angiotensin and sympathetic nervous systems, and antidiuretic hormone are the common pathophysiological pathways, but the origin is different. Platelets are known to have a key role in the mechanisms associated with pre-eclampsia. These similarities suggest the possible role of platelets in the development of OHSS.

In a recent study, the clinical and laboratory data of 13 patients with OHSS were analysed retrospectively and compared with the same number of patients (selected by matching age, body weight, parity) without OHSS. In all cases, ovarian stimulation was performed using combined suppression/stimulation therapy in an in-vitro fertilization and embryo transfer programme at the Womens Hospital, University of Tübingen, Germany. The gonadotrophin-releasing hormone agonist triptorelin (Decapeptyl®; Ferring, Kiel, Germany) was used in a long protocol. The stimulation was performed with individual dosages of human menopausal gonadotrophin (Humegon®; Organon, Oberschleissheim, Germany), varying from two to six ampoules depending on the follicular maturation. Ovulation was induced by injection of 10 000 IU human chorionic gonadotrophin (HCG, Predalon; Organon), and aspiration of follicular fluid was performed 36 h later by ultrasound-guided vaginal puncture. All women received between one or three cleaved oocytes at embryo transfer 2 days later. Hormonal support with 2×5000 IU HCG was given to all women if no signs of OHSS were present.

Table I. Clinical and laboratory data of patients with or without ovarian hyperstimulation syndrome (OHSS)

	OHSS group	Control group
Number of patients	13	13
Age (years)	31 (25–42)	31 (26–42)
Height (cm)	158 (152–173)	157 (151–174)
Weight (kg)	55 (49–76)	55 (48–76)
Cycle length (days)	28 (24–34)	28 (24–34)
HMG (days)	10 (8–14)	
HMG (ampoules)	29 (17–46)	28 (17–45)
Cleaved oocytes	4 (2–8)	4 (1–8)
Embryos transferred	3 (2–3)	3 (2–3)
Pregnancy	6	4
Platelets		
before stimulation	223 000[a] (183–248)	186 000 (168–210)
at OHSS	411000[a] (371–610)	196 000 (174–221)

HMG = human menopausal gonadotrophin.
[a]Significant difference ($P < 0.001$) between these two values.

The important clinical and laboratory data are summarized in Table I. There were significant differences between the two groups in platelet counts ($P < 0.001$). Other clinical and laboratory data were very similar and without significant differences in the two groups.

According to current knowledge, platelets are very powerful particles that can actively store, release, and in part synthetize some biologically very effective materials. It seems that they have an important role in the oocyte maturation. Murdoch (1986) observed platelet aggregation and adhesion to the endothelial cells in the capillaries around the periovulatory ovine follicles. Li *et al.* (1991) reported that platelets are activated by platelet-activating factor (PAF) during gonadotrophin-induced ovulation. Platelets can release various constituents, including histamine, serotonin, and platelet-derived growth factor. Normally these pathways do not require systemic compensatory mechanisms because of physiological control; however, during superovulation treatment ovarian stimulation is not under the control of physiological regulation. The enormously amplified endocrine, metabolic, and circulatory changes associated with platelet hyperstimulation secondarily induce the compensatory mechanisms which appear as well known clinical symptoms.

Our results suggest that the higher number of platelets may be responsible for some symptoms associated with OHSS. We propose that specialists might consider control of platelet count and activity in cases of ovarian hyperstimulation syndrome.

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Dear Sir,

We thank Bodis *et al.* (1998) for their remark and interesting observation of increased platelet count in 13 cases of ovarian hyperstimulation syndrome (OHSS). In order to elaborate this issue we performed a survey of the platelet count of 35 patients with severe OHSS upon admission to our Obstetrics and Gynecology Departments in Hadassah University Hospital in Jerusalem between 1987–1997. The mean ± SD of platelet count on admission was found to be 340 370 ± 112 550 (median 328 000) with range of 162 000–650 000. The initial count was found to be within the normal limits (150 000–400 000) in 25 out of 35 patients (71%). A total of 10 patients (29%) had platelet counts above the normal limits with a mean of 485 000 (414 000–650 000) and three patients had a platelet count >500 000. Two patients had thromboembolic phenomena, one with 245 000 thrombocytes and the other with 414 000 thrombocytes.

Our data differs from the data presented by Bodis *et al.* (1998). The mean platelet count in our group was found to be within the normal limits (340 370 in comparison with 411 000 found by Bodis *et al.*). Although, only severe cases of OHSS were included in our group (whereas Bodis *et al.* did not mention the severity of OHSS in their cases), in our homogeneic severe OHSS group, 71% of the patients had normal platelet count. If we were to believe that increased platelet count has a key role in triggering or promoting OHSS, then the thrombocyte count should have been increased consistently above the normal limits in every patient with severe OHSS. However, our data fail to show this kind of relationship. It is known that circulating plasma concentrations of immunoreactive endothelin, vascular endothelial growth factor and various other cytokines are elevated in patients with severe OHSS in parallel with other neurohormonal/vasoconstrictor systems according to the severity of the syndrome (Abramov *et al.*, 1996, 1997). It is also known that in severe OHSS leukocyte count and haematocrit are increased secondary to the haemodynamic changes which leads to third space fluid accumulation and haemoconcentration. According to our observation it seems that the relative thrombocythaemia in OHSS is not consistent and probably is a secondary phenomenon rather than a triggering factor initiating OHSS. However, we agree that thrombocytemia may be responsible for some symptoms such as thromboembolic phenomena that occur rarely in severe cases of OHSS.

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