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MEETING REPORT

Cigarette Smoking and Fertility

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Abstract: A strong body of evidence indicates that the negative effect of cigarette smoking on fertility comprises fairly every system involved in the reproductive process. The impact of cigarette smoking on ovarian reserve is clearly evidenced by younger age at menopause of smokers. Tobacco compounds' impairment of the process of ovarian follicle maturation is expressed by worse in-vitro fertilization parameters in cycles performed on women with smoking habits. Also, uterine receptiveness and tubal function are significantly altered by the smoking habit. In men, cigarette smoking reduces sperm production, increases oxidative stress, and DNA damage. Spermatozoa from smokers have reduced fertilizing capacity, and embryos display lower implantation rates. Couples at reproductive age should be strongly discouraged to smoke.

Keywords: fertility, tobacco, smoke esposure

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Introduction

A thorough review of the literature on cigarette smoking and fertility identifies strong evidence of an association between tobacco smoke exposure and impaired reproductive function. However, the conclusions have not been as unanimous as expected. One aspect that is readily evident in such a review is the general statement that potential mechanisms by which smoking may impair fertility are varied, with none being clearly established. Indeed, in some cases, an association between two variables is recognized but in the majority of cases causality is not easily established.

Factors which aid in identifying causality are, among others: the strength of the association between variables, the specificity of this association, the existence of temporal sequence between exposure and the documentation of the effect and the existence of biological plausibility.Concerning cigarette smoking and fertility, the particular aspects related to these factors are:

a) *Strength of the association*: if the exposure to a certain environmental factor impairs a certain physiological step or function, but, in general, not very strongly, this means that the statistical documentation of this impact demands studies with larger sample sizes. This does not mean the association is not clinically relevant, especially if we consider that a final outcome (for example, reduced fertility) may be the consequence of the coexistence of a number of moderately impaired physiological steps. Studies on sperm production, for instance, make this issue particularly relevant and will be dealt with later.

b) *Specificity of the association*: the lack of specificity of a certain association means that other variables interfere with the outcome being studied. This fact imposes a certain methodological care in study design. In the case of reproductive function, it is known that female age, BMI, partner's smoking status and diseases that affect reproductive function must be controlled variables.

c) *Temporal sequence*: regarding temporal sequence, studies of toxic exposure in humans have, very often, obvious methodological limitations in the control of time of exposure.

d) *Biological plausibility*: biological plausibility for the association between tobacco and impaired fertility is abundant and constantly reinforced.

Studies may be performed in animals and humans. The purity of the information in terms of a dose-response effect, the lack of bias and temporal sequence is higher in studies with animals, but extrapolation of information from studies to the clinical setting is more direct in studies with humans.

Therefore, when all these obstacles are gathered in the search of clear evidence of the effect of smoking in the reproductive function, the scenario is certainly complex and the analysis of available information will expose all the difficulties and limitations that are faced and that may also vary from one analysed target organ to another.

In spite of all this, there are some consistent findings and reviews which support the thesis that a significant correlation exists between tobacco smoke and altered reproductive physiology.

Cigarette Smoking and Female Fertility

In women, the association between smoking and the incidence of infertility was the subject of a meta-analysis published in 1998.[1] This review found a significant increase in the incidence of infertility among smokers when the information from the 12 studies was collected and the consistency of effect across different studies was very persuasive. Two years before, a systematic review of published studies of the same issue had been carried out, but data were not gathered. Some of these studies were later included in the meta-analysis we have just mentioned, but even when we consider only those that were not, we again see a high consistency in the association between smoking and reduced fecundity.[2]

Cigarette smoking and ovarian function

Among all reproductive system targets, ovarian tissue is by far the most widely studied in terms of the consequences of exposure to tobacco compounds. Chemicals in cigarette smoke appear to accelerate follicular depletion. Menopause occurs 1–4 years earlier in women who smoke and an increased incidence of POF is also documented.[3–5] Basal FSH levels were reported to be 60%–70% higher in active smokers than in non-smokers and 40% higher in passive smokers than in non-smokers.[6]

In parallel to this quantitative effect on follicles, a qualitative effect on steroidogenesis, follicular/oocyte

maturation, fertilization and embryo development has also been documented. Information accumulated in recent years indicates that oxidative stress plays a significant role in the pathophysiological processes involved in fertility reduction related with the quality of both gametes. It is known that reactive oxygen species are capable of damaging every molecule present inside the cell: carbohydrates, proteins, lipids and the DNA. Therefore many of the mechanisms of impaired reproductive function mentioned may ultimately be related, to some extent, to oxidative stress.

Steroidogenesis

In vitro studies have shown that high cadmium concentrations inhibit the expression of the Cytochrome P450 side chain cleavage gene and therefore, aromatase activity[7] and several clinical studies[8,9] confirm that cigarette smoking has an antiestrogenic effect in women. Another possible explanation for lower estradiol levels seen in smokers may be the increased rate of 2-hydroxylation.[10] Higher conversion rate to catecolestrogen could reduce the availability of estradiol. Cotinine, the main metabolite of nicotine, has also been linked to granulosa-luteal cell function inhibition through an anti-mitotic and apoptotic effect, being a possible determinant of luteal insufficiency.[11]

Follicle/oocyte maturation

In vitro studies have shown that high concentrations of cadmium and nicotine impair cumulus expansion by respectively, suppressing hyaluronic acid synthesis and release.[12]

Tobacco exposure was shown to alter the meiotic spindle of ooyctes in women, leading to disjunctional errors.[13–15] Studies in animals confirm alterations in the meiotic spindle assembly and chromosome alignment. When we consider that studies of smoking patients undergoing ART show a significant decrease in the number of oocytes retrieved, we may hypothesize that this may be the expression of the chronic process of accelerated ovarian reserve consumption in smokers, but may also be the consequence of short-term effects of tobacco compounds in the process of meiotic maturation, leading to meiotic arrest.

A suspicion of altered follicular vascularization in smokers also exists, since the concentration of soluble VEGF receptor-1 was shown to be increased,[16] but this effect is not clear.

Fertilization and embryo development

In animals, exposure to tobacco compounds adversely affects pre and post-implantation embryo developmental competence.[17–19] Smoking inhalation by the female adult mouse is detrimental to early embryo development and leads to reduced fetal body weight. Direct injection of nicotine in rats retards embryo cleavage and substantially reduces embryo cell number. A time and dose effect was observed.

Genetic effects of tobacco compounds exposure include reduced blastocyst DNA synthesis and telomere shortening and loss.[20,21]

IVF cycles outcome

Several studies investigated if a smoking habit influences IVF outcome.[22,23] The lack of consensus among studies reflects the methodological difficulties mentioned in the beginning of this presentation. Clear sources of bias are the variations in the definition of cigarette smoking and not controlling for male partner smoking history.

The majority of the studies report lower peak estradiol levels, fewer oocytes retrieved, the need of higher doses of gonadotrophin and longer stimulations. Effects in fertilization and embryo morphology are particularly controversial.

Clinical outcomes, such as clinical pregnancy rate (CPR), miscarriage rate and live birth rate have been approached by using meta-analysis.[1,24,25] All published meta-analyses found a significant reduction in CPR and, very interestingly, to a very similar degree. Pregnancy probability of an IVF cycle performed on a smoking woman seems to be around 60% that of a non-smoker. The most recent meta-analysis, including 21 studies, also demonstrates a significant increase in miscarriage rate and reduced live birth rate in smokers.[25] Ex-smokers have better chances of success.[2]

Cigarette smoking and tubal function

Data regarding the effects of smoking on tubal function in animals show that, in rodents, exposure to cigarette smoke or just nicotine can interfere with ciliary beat frequency of the tubal endothelium, oocyte pick-up and infundibular smooth muscle contractions and can

cause an increase in the infundibular secretory-to-ciliated cell rate.[26–28]

In humans, it is documented that women who smoke have an increased incidence of tubal infertility and a markedly increased risk of ectopic pregnancy.[29] Compared to women who have never smoked, those who smoke more than 20 cigarettes a day have an almost four-fold incidence of ectopic gestation, a risk that is similar to that observed in patients with a past history of pelvic inflammatory disease. These data refer to spontaneous pregnancies, but the recent meta-analysis on female smoking and IVF outcome[25] also studied this issue in IVF cycles. The OR of an ectopic pregnancy among smokers was 15.69 (CI 2.87–85.76). Cigarette smoking has been demonstrated to adversely affect both the humoral and cellular immune systems. It is speculated that such alterations may make smokers more prone to tubal infections and subsequent infertility.

Cigarette smoking and uterine function

Although the existence of an effect of cigarette smoking in the ovarian function has long been known, until recently, no direct evidence had been accrued to confirm a concomitant uterine effect. The hypothesis of an impact of tobacco compounds on uterine receptiveness was raised by indirect evidence from in vitro and in vivo studies:

a) Loss of cell adhesion and reduced basement membrane invasion were observed in human endometrial adenocarcinoma cell cultures exposed to tobacco constituents (benzo(a)pyrene).[30] This cell line serves as a model for receptive endometrium due to its adhesiveness for trophoblast cells.
b) In the clinical field, increased resting uterine tonus in female smokers was described decades ago.[31] In addition, lower endometrial and subendometrial vascularity and flow intensity throughout the menstrual cycle were reported in smokers,[32] although no such effect was detected during IVF treatments.[33] Finally, some authors reported lower pregnancy and implantation rates in smokers undergoing IVF cycles in which the number of morphologically good embryos replaced was similar to that seen in nonsmokers.[34] This suggested that altered uterine receptiveness might be a factor in the former group of women, although reduced implantation could also be due to embryonic factors that belie a good morphology on day 3 of development.

Oocyte donation is the model that most objectively ascertains the presence of a uterine factor in the outcome of assisted reproduction cycles. Recently, our group studied the clinical impact of oocyte recipients' tobacco consumption in the outcome of these cycles.[35] 785 embryo transfer cycles were analysed. Husband and donor smoking status were controlled variables, as well as donor and recipient age, recipient BMI and embryo number and quality. Pregnancy rate in heavy smokers was significantly lower. Unexpectedly, a significant increase in the incidence of twin gestations in heavy smoking oocyte recipients was also observed. The association of reduced pregnancy rate and increased multiple pregnancy rate (MPR) in smokers is paradoxical. The increased incidence of MPR among smokers is very intriguing and deserves further investigation. It is noteworthy that a high MPR in heavy smokers had been previously described in spontaneous pregnancies,[36,37] but mechanisms considered responsible for this finding (polyovulation due to hormone disturbances or zona pellucida hardening leading to embryo splitting during hatching) cannot explain the same finding in the context of oocyte donation. Interestingly, a significant reduced pregnancy rate and increased dizygotic twin gestation rate in IVF cycles performed on couples in which either the male or the female partners were heavy smokers had also been previously described.[38] The same finding observed in oocyte donation[35] suggests that the underlying mechanism is related to the uterus, rather than the gametes.

Cigarette Smoking and Male Fertility

Studies of natural conception in couples with a smoking male partner demonstrate a significant reduction in fecundity, with an increased time-to-pregnancy, only when tobacco consumption is above 15 cigarettes a day.[39,40]

Cigarette smoking and classical sperm parameters

Literature on the association between cigarette smoking and classical sperm parameters is conflicting, in some cases clearly due to a sample size that is not large

enough to detect differences that may be of clinical relevance, in others due to the lack of control over a dose-response relationship or confounding variables. The largest cross-sectional study on this issue was published in 2007.[41] 2,542 healthy males had their sperm samples analysed and it was shown that with increasing smoking, a 20%–30% reduction in sperm count, volume and motile spermatozoa was observed. These figures may explain why studies with small sample sizes do not detect statistical significance in the reductions in sperm parameters in smokers. Also, they may elucidate the modest clinical relevance of a smoking habit in cases of a good primary testicular function.

In cases of infertility, however, it must be borne in mind that, apart from the alterations in classical sperm parameters, tobacco compounds may affect sperm quality in other ways. Biochemical changes that may reduce sperm quality have been documented in seminal fluid of smokers and genotoxicity of tobacco smoke is indisputable.

Cigarette smoking, oxidative stress and sperm abnormalities

Oxydative stress occurs in the seminal fluid of male smokers: the concentrations of cadmium, lead, reactive oxygen species (ROS) and others are significantly higher[42] and, at the same time, the concentration of ascorbic acid and the activity of other components of the antioxidant defense are significantly reduced.[43] Ascorbic acid is the main extracellular water-soluble antioxidant. The scavenging capacity of the antioxidant defense system is, therefore, limited.

Physiological and structural abnormalities

Oxidative stress has been linked to a number of physiological and structural abnormalities in human sperm. Fertilizing capacity is reduced due to failure to extrude residual spermatozoon cytoplasm, diminished membrane maturation and acrosin activity and an increased incidence of structural abnormalities in sperm tail.[44–46] Cotinine has been targeted as one relevant molecule on the impairment of these processes. Spermatozoa cultured in a medium enriched with this molecule have a significant reduction of hyperactivation and worsened membrane function evaluated by the hypoosmotic swelling test and the capacity of penetrating zona-free hamster oocytes.[47] Interestingly, the capacity of micro-injected sperm to activate hamster oocytes is maintained.

The DNA damage caused by ROS is documented and constitutes the worst effect of oxidative stress in sperm.

Genetic alterations

- Polycyclic aromatic hydrocarbons metabolites link to nitrogenated base guanosine and form premutational/carcinogenic lesions known as DNA adducts. The incidence of DNA adducts is significantly higher in spermatozoa of smokers.[48] Their presence raises the issue of the risk of increased germ-line heritable DNA sequence mutations. In mice, an increased incidence of mutations was documented in spermatogonial cells of animals exposed to tobacco smoke.[49] Data suggested that mutations accumulate with extended exposure. In humans, paternal smoking habit was associated with an increased rate of DNA adducts in day 3 embryos[50] and an increased incidence of childhood cancer,[51] a condition known to be linked to genetic mutations.
- Different groups studied sperm DNA fragmentation in smoking and nonsmoking men from infertile couples. An increased rate of fragmentation in smokers was found in pre and post swim-up samples.[52–54] This finding is associated with reduced embryo quality in IVF cycles.[55,56] Interestingly, young, healthy, fertile smoking donors were reported not to display a significant increase in DNA fragmentation in spermatozoa.[47,57] This may be the consequence of a more efficient protection against ROS or a more efficient DNA repairing machinery during the immature germ cell phase.
- Results of studies that investigated the incidence of aneuploidy in sperm of smokers have not been uniform. An increased incidence of aneuploidy rate for some chromosome pairs was documented in smokers, namely for pairs 1 and 13.[58,59] This is an issue that must be analysed more deeply, but susceptibility to smoking induced non-disjunction seems to vary among chromosome pairs and individuals.

IVF cycles outcome

Humans

Based on data previously mentioned, studies tried to clarify if male tobacco consumption worsens

the prognosis of IVF cycles. Couples with a male smoker were reported to have reduced fertilization rate, implantation rate, pregnancy rate and a reduced probability of achieving a 12-week gestation,[60–63] but literature that investigated specifically the impact of male smoking in IVF parameters is poor. The identification of a number of abnormalities in the genetic material of spermatozoa produced by smokers indicate that, even if ICSI overcomes many of the drawbacks in sperm function determined by tobacco exposure, the presence of DNA adducts, DNA fragmentation and maybe an increased aneuploidy rate are likely to impair IVF cycle prognosis. Indeed, a significantly lower pregnancy rate with either conventional IVF or ICSI was reported in couples with a smoking husband.[62]

The probability of achieving a clinical pregnancy by performing testicular biopsy in cases of azoospermia was also shown to be related to a smoking habit.[64] Smoking azoospermic men have a reduced probability of a clinical pregnancy (17.4 vs. 43.7 in the study published) and live birth (20% vs. 43.2%) when compared to azoospermic non-smokers. The dramatic contrast of these figures reinforces the concept that the impairment in testicular function determined by tobacco exposure depends highly on the quality of primary testicular physiology, a concept that was already raised by the observation that young, healthy, fertile smoking donors do not have increased sperm DNA fragmentation.

Animals

In findings of IVF cycles performed on couples with a male smoker, it is difficult do differentiate between a direct sperm effect of tobacco consumption and all the possible effects of passive smoking on the female partner. This difficulty is clearly illustrated by the report of reduced number of oocytes retrieved in IVF cycles performed to partners of male smokers.[65] The animal model allows for an absolute control over confounding variables such as passive female smoking and male exposure to other toxicants.

In rats, male exposure to cigarette smoke results in a secretory deficiency of Sertoli and Leydig cells and impaired epididymal maturation.[66] In vivo fertility and in vitro fertilizing capacity with conventional IVF are reduced. ICSI is capable of overcoming the in vitro limitation in fertilization and early embryo development is apparently normal, but reduced implantation potential of embryos obtained from smoking male rats is observed regardless of the insemination technique used (IVF or ICSI).

In conclusion, a strong body of evidence demonstrates that the exposure to tobacco constituents affects sperm function and integrity. Some of the drawbacks identified seem to be overcome by ICSI, but genetic damage would still compromise embryo implantation potential. Cessation of smoking can increase the potential of achieving pregnancy.[67]

Summary

Cigarette smoking reduces female fertility natural cycles as well as in assisted reproductive cycles. A quantitative and qualitative effect is observed in ovarian function. The incidence of tubal infertility and tubal pregnancy is increased (this last one, in spontaneous and IVF pregnancies). Uterine receptiveness is altered. Male fertility is compromised in heavy smokers and classical sperm parameters are moderately reduced. Biochemical alterations are seen in seminal fluid and the incidence of DNA damage is increased in sperm from smokers. Embryos generated from sperm of smokers have reduced implantation potential.

Disclosure

The authors report no conflicts of interest.

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