J Clin Periodontol 2011; 38: 542–546 doi: 10.1111/j.1600-051X.2011.01720.x

Journal of Clinical Periodontology

Periodontal status of males attending an in vitro fertilization clinic

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Klinger A, Hain B, Yaffe H, Schonberger O. Periodontal status of males attending an in vitro fertilization clinic. J Clin Peridontol 2011; 38: 542–546. doi: 10.1111/j.1600-051X.2011.01720.x.

Abstract

Aims: An association has been demonstrated previously between periodontal diseases and various systemic conditions, including endometriosis. A possible role of dental infection in male infertility was also suggested. The aim of the present study was to examine the association between fertility parameters and the periodontal status of men attending a fertility and in vitro fertilization (IVF) clinic.

Methods: The study population consisted of 75 men attending the clinic for sperm analysis before homologue semen insemination or IVF. The quality of sperm was assessed according to WHO criteria. On the same day, patients received a clinical periodontal examination.

Results: The patients were diagnosed with either gingivitis (40%) or periodontitis (48%), whereas the remaining 12% were classified as ''periodontally healthy''. Normospermia was attributed to 37%, oligozoospermia to 48% and azoospermia to 15% of these patients. Familial infertility was significantly associated with having at least one WHO parameter contributing to infertility. A higher number of sites with deep periodontal pockets tended to associate positively with sperm sub-motility. Clinical attachment levels were significantly associated with sperm sub-motility.

Conclusions: These findings may point to a possible association between male infertility, diminished semen quality and periodontal infections in men attending fertility and IVF clinics.

Key words: clinical attachment level; fertility; gingival inflammation; periodontitis; sperm motility

Accepted for publication 24 February 2011

Infertility, which affects 15% of all couples worldwide, is defined as the failure of a couple to conceive after 1 year of unprotected intercourse (WHO 1999). In about half of all cases, infertility is attributed to the male partner (male factor) and results from poor sperm quality. Male factor includes one or more of the following: low sperm production (oligozoospermia), poor sperm motility (asthenozoospermia) or abnormal sperm morphology (teratozoospermia) (Guzick et al. 2001). In many cases, infertility of the male partner can be traced to overt infections, immunologic problems, hormonal imbalance, anatomic defects, ejaculatory failures and environmental exposure. In about half of the cases, however, the aetiology of male infertility remains unexplained.

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
There was no external source of financial support or funding.
Clinical trial registration number: NCT01140945

Remote, focal infections are attractive candidates to explain some of these cases because such infections release immunological mediators and modify the overall health state of the patient. Since the historical paper of Miller (1891) on focal infection, numerous studies dealing with the possible interactions between oral infections and various systemic conditions have been published (DeStefano et al. 1993, Joshipura et al. 1996, Beck et al. 1998, Wu et al. 2000, Soskolne & Klinger 2001, Offenbacher et al. 2009). However, the possibility of an association between fertility in a man and his oral health was only scarcely addressed in the medical literature. Bieniek & Riedel (1993) published a case series suggesting a direct causal relationship between dental bacterial infections (foci) and therapy-resistant bacteriospermia, perhaps leading to sub-fertility.

Kavoussi et al. (2009) investigated whether an association exists between endometriosis and periodontal disease in a cross-sectional study design. Patient

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data comprised 4136 women, ages 18–50, in the National Health and Nutrition Examination Survey, 1999–2004. Women with self-reported endometriosis had significantly higher odds of having both gingivitis and periodontitis to not having periodontal disease, compared with women without self-reported endometriosis.

In generally healthy men, periodontal diseases are by far the most common focal infection, which often remain covert and therefore untreated, and yet, the inter-relation between male fertility and periodontitis was never investigated. The aim of the present study was to examine whether an association exists between sub-fertility and periodontal status in men attending fertility and in vitro fertilization (IVF) clinics.

Materials and Methods

The study was approved by the institutional review board (IRB) for Human Studies of the Hadassah Medical Center, Jerusalem, Israel, and in accordance with the World Medical Association Declaration of Helsinki (version VI, 2002) and all participants signed the consent form, which was approved by the IRB. The study population consisted of 75 male spouses in infertile couples attending our IVF clinic from October 2007 to August 2009. Study participants underwent sperm analysis for preparation before homologue semen insemination or IVF. The examinations included sperm analysis, followed by a clinical periodontal examination, without performing X-rays. The periodontal clinical parameters recorded were number of missing teeth, full-mouth plaque score, number of teeth with gingival index >0, number of sites with bleeding on probing (BOP), number of mobile teeth, number of sites with probing depth (PD) >3 mm, number of sites with recession >1 mm and number of sites with clinical attachment levels (CAL) >1 mm. Periodontal diagnosis was classified as either healthy, gingivitis (at least two sites with gingival index >0, without attachment loss) or periodontitis (presence of at least two sites with true periodontal pockets of 4 mm or deeper with BOP).

Semen was collected, analysed and classified according to the criteria of the World Health Organization (WHO 1999). Semen was obtained by masturbation into sterile plastic containers or after ejaculation into a sterile medical non-spermicidal condom following 3 days of abstinence. Semen analyses were performed within 30 min. of sample arrival in the laboratory. Sperm parameters recorded were ejaculate volume, sperm cell concentration, motility (overall, progressive), morphology and the presence of white cells. Patients were asked to report the existence of familial infertility. Smoking and antibiotic intake during the past year was also recorded. Fertility was classified as either fertile or sub-fertile, according to three main parameters: sperm cell concentration, sperm cell motility and sperm cell morphology. Normozoospermia is attributed to normal ejaculate as defined by the WHO reference values. Normal ejaculate constitutes a sperm cell concentration of 20 million sperm/ml or greater, with at least 25% rapid progressive motility and 30% or more normal morphology. Oligozoospermia occurs when the sperm concentration is less than the reference value of 20×10^6/ml; azoospermia occurs when there are no spermatozoa in the ejaculate. Sperm submotility is sperm pathology when $<25\%$ spermatozoa are classified as having a rapid progressive motility.

It is widely accepted that patients having abnormal values in one of the sperm measurement parameters also have two to threefold higher odds of being infertile. In patients with abnormal values of two of the tested parameters, the odds of infertility increase by five to sevenfold, while patients having abnormal values of three parameters have 16-fold chances of being infertile (Guzick et al. 2001).

Statistical analysis

ANOVA multivariate analysis and Yates χ^2-test were used for examining a possible association between the tested periodontal and fertility parameters. Controlling for confounding factors such as age, smoking, familial infertility and antibiotic intake during the past year was performed. The primary outcome measure of the present study was a positive association between periodontitis or gingivitis and diminished sperm counts. The secondary outcome measure is positive associations between periodontal parameters and sperm sub-motility or altered sperm morphology.

Results

Data were available for the 75 examined subjects (Tables 1 and 2). The proportion of subjects with periodontitis was 36/75, representing 48% of the examined subjects, surprisingly higher than those with gingivitis 30/75 (40%). Normospermia was diagnosed in 28/75 of the patients and oligozoospermia in 36/75. Sperm sub-motility was diagnosed in 23/75, and abnormal morphology in 10/75 of the subjects, representing 13.33%. Figure 1 shows the overall distribution of study participants according to their periodontal diagnosis and sperm count. Most of the subjects examined were diagnosed with gingivitis or periodontitis and were either normospermic or oligospermic. Inter-group comparisons showed that the differences between groups were not statistically significant. No significant association could be demonstrated between periodontitis or gingivitis and the diagnosis of infertility based on the observed sperm counts (ANOVA, $p = 0.17$).

The secondary outcome measure was a possible association between periodontal and other fertility parameters. Figure 2 demonstrates that a higher percentage of subjects with one abnormal fertility parameter have also reported a familial infertility (89%) compared with subjects who have not (67%). The difference between these groups was statistically significant (χ^2, $p = 0.04$).

Table 3 shows the distribution of motile and sub-motile subjects accord-

Table 1. Basic demographics of the studied population

Age	32.7 years
Familial infertility	21/75 (28%)
Smoking	15/75 (20%)
Antibiotic intake during past year	29/75 (38.7%)

Smoking status is expressed as the ratio of subjects who reported smoking more than 1/2 pack/day during the last 5 years. Antibiotic intake is expressed as the ratio of subjects who reported intake of antibiotics during the past year.

Table 2. Study epidemiology according to periodontal and fertility diagnoses

Periodontal diagnosis	
Healthy	9/75 (12%)
Gingivitis	30/75 (40%)
Periodontitis	36/75 (48%)
Fertility diagnosis	
Normospermic	28/75 (37%)
Oligozoospermic	36/75 (48%)
Azoospermic	11/75 (15%)
Sub-motility	23/75 (30.7%)
Abnormal morphology	10/75 (13.3%)

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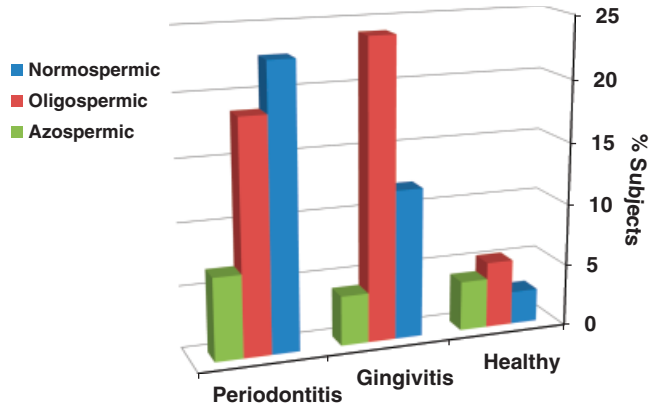

Fig. 1. The distribution of study participants according to their periodontal diagnosis and sperm counts.

Table 3. Distribution of subjects with motile or sub-motile spermatocytes according to their periodontal parameters

	Sub-motility (SD)	Normal motility (SD)
Age	33.7 years (6.7)	31.2 years (6.9)
#Missing teeth	0.5 (1.5)	0.6 (2.0)
Plaque-score (%)	38.2 (27.9)	44.1 (26.2)
#Teeth GI >0	11.0 (10)	11.3 (9.5)
#BOP sites	6.0 (9.9)	7.0 (15.2)
Degree of mobility	0.2 (0.7)	0.3 (0.7)

No statistically significant difference was found between groups (multivariate ANOVA, $\alpha = 0.05$).

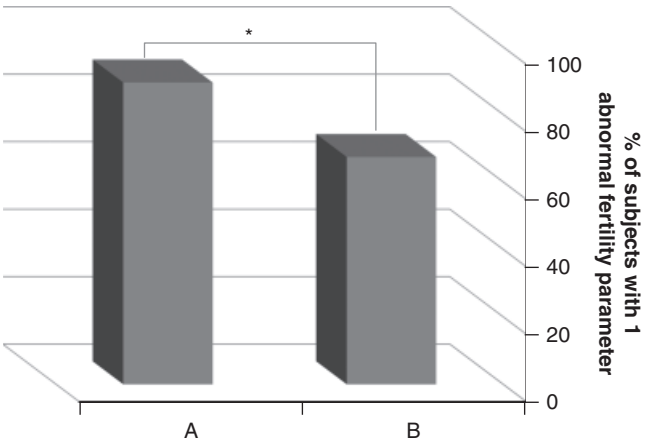

Fig. 2. Familial infertility and an abnormal fertility parameter. (A)–percentage of subjects with familial infertility and one abnormal parameter; (B) percentage of subjects without familial infertility and one abnormal parameter. *Statistically significant difference between groups (χ^2 analysis, $p = 0.04$).

ing to their periodontal parameters. No statistically significant difference was found between these groups in age, plaque score, number of missing teeth, number of teeth with a gingival index of 1 or higher, number of sites with bleeding upon probing and the mean degree of tooth mobility. Overall, heavy smoking was reported by 20.0% of the study population (Table 1). No statistically significant difference, however, was found between the percentage of smokers in the motile compared with the sub-motile subjects.

Figure 3 shows the distribution of the subjects according to their sperm motility status and periodontal diagnosis. In the motile group, more of the patients were classified with a healthy periodontium as opposed to those with gingivitis or periodontitis. On the other hand, a higher tendency for periodontitis could be observed in the sub-motile patients. This tendency was further emphasized when the threshold for periodontitis was set to three sites or more with PD > 3 mm (B) instead of two sites (A). In this type of comparison, the ratio of the sub-motile/motile patients was approximately 1/4 in the periodontally healthy group, as compared with more than 2/4 in the periodontitis group.

Figure 4 demonstrates a significantly higher percentage (95.45%) of subjects with one site or more with CAL > 1 mm in the sub-motile group, compared with the motile group (68.75%). This difference was statistically significant (Yates $\chi^2 = 4.681$, $p = 0.03$).

Discussion

The findings presented herein provide a proof of concept for the investigated interaction of two systemically distinct conditions. As the gold standard logistic regression step-wise analysis was not used because of sample size limitations, we performed exploratory analyses using the methods described.

The proportion of subjects with periodontitis in the present study was found to be 36/75, representing 48% of the examined subjects, similar to the expected worldwide figure (Albandar 2005), but surprisingly higher than those with gingivitis 30/75 (40%). Estimates of the general prevalence of adult gingivitis vary from approximately 50% to 100% for dentate subjects (Ericsson et al. 2009). Oligozoospermia was diagnosed in 36/75 of the examined subjects. As to other fertility parameters, sperm sub-motility was diagnosed in 23/75 and abnormal morphology in 10/75 of the examined subjects. These numbers fit worldwide accepted figures for fertility clinic patients (Guzick et al. 2001).

As for the primary and secondary outcome measures investigated in this study, it would be difficult to compare our findings with those of others mainly, as this pilot study investigated an association between two systemically distinct conditions that until to date were not linked together.

However, various mechanisms of action could be considered as possible explanations for the observed association. Toth & Lesser (1981) examined bacterial flora of the seminal fluid in asymptomatic men. Fertile men had significantly fewer positive cultures than any other group selected from the infertile population. Significantly more bacterial isolates were obtained from patients with a history of genital tract

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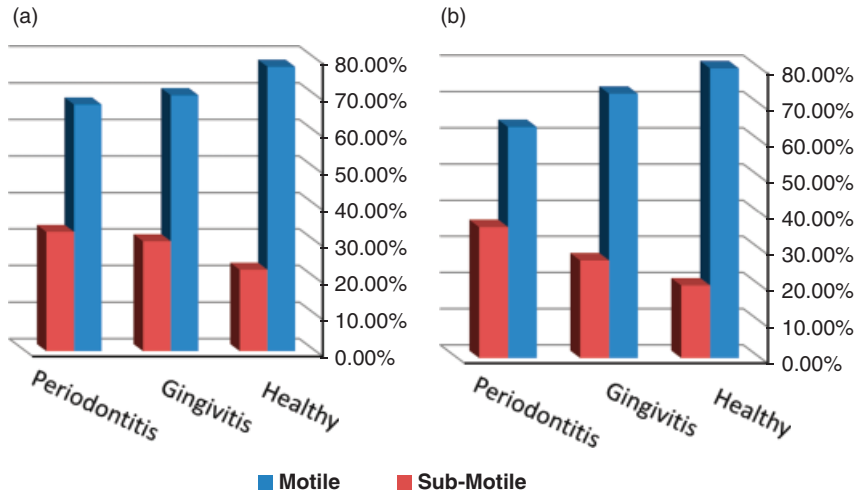

Fig. 3. Distribution of subjects according to their motility status and periodontal disease, diagnosed on the basis of number of sites with probing pocket depth (PD) >3 mm. (A) Periodontitis = two sites or more with PD>3 mm. (B) Periodontitis = three sites or more with PD>3 mm.

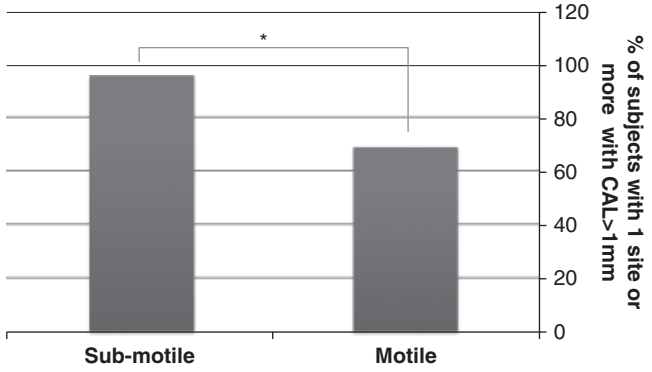

Fig. 4. Sperm motility and percentage of subjects with sites that lost clinical attachment level. *Statistically significant difference between groups (Yates $\chi^2 = 4.681$, $p = 0.03$).

infection than from infertile men without a history of genital tract infection. Jarvi et al. (1996) have shown over 10^4 bacteria/ml in the semen of 66% of the infertile asymptomatic men and 66% of the semen donors using PCR. This contrasts with routine culture results, which detected ''significant'' bacteriospermia in only 27% of the infertile men and in none of the preselected semen donors. From four of these semen specimens, DNA sequence analysis identified an average of nine different bacterial species per specimen, with close to 90% of the species being anaerobes.

Bieniek & Riedel (1993) implied that bacteriospermia might be initiated from oral foci of infection spreading through the circulation. However, this is widely disputed mainly because of a possible contamination of the seminal fluid by the bacterial sampling technique, yielding a false-positive test result (Kim & Goldstein 1999).

Offenbacher et al. (1996) contemplated that cytokine production involved in the pathogenesis of periodontitis may also be involved in various systemic conditions such as coronary heart disease and preterm lower birth weight.

Naz & Kaplan (1994) showed the presence of various cytokines, namely tumour necrosis factor (TNF-α), interferon (IFN-γ) and interleukin (IL)-1β, in seminal plasma of fertile, infertile and immunoinfertile men using specific immunoradiometric assays. IL-6 was detected in seminal plasma with significantly higher levels in infertile/immunoinfertile men compared with those of fertile men. IL-6 was also present in the sera, and interestingly, the levels in the sera were lower than those in seminal plasma. IL-6 levels in seminal plasma correlated significantly with some sperm parameters and penetration rates in the human sperm penetration assay (SPA). These findings suggested that IL-6 is associated with infertility and may be of importance in the specific diagnosis and treatment of male (Naz & Kaplan 1994, Huleihel et al. 1996, Camejo et al. 2001, Matalliotakis et al. 2006) or female (Kavoussi et al. 2009) infertility. A recent study by Offenbacher et al. (2009), however, questioned the role of elevated plasma levels of cytokines as a possible causative factor in the preterm low birth weight.

Heat shock proteins (HSPs) are proteins presented on vascular endothelium in response to elevated body temperature, oxidizing agents and lipids. Resemblance in the antigenicity of bacterial and human HSPs has been demonstrated. Maeda et al. 1994, showed that *Porphyromonas gingivalis* possesses a HSP. Antibodies (Ab) against this HSP that are cross reactive with a human HSP.

Eggert-Kruse et al. (2002) studied the role of sensitization to HSP and its potential role in the aetiology of male infertility. The potential association of immunoglobulin (Ig)A Ab with the human HSP 60 with several parameters of sub-clinical male genital tract infection/inflammation and with semen quality and sperm fertilizing capacity was analysed in a prospective study. IgA Ab to human HSP 60 were determined in seminal plasma of randomly chosen male partners of sub-fertile couples with a median duration of infertility of 4 years, who were asymptomatic for genital tract infection. They demonstrated that the presence of HSP 60 IgA Ab in seminal fluid was significantly associated with leukocytospermia and also with high interleukin levels in seminal plasma. Their findings might suggest a potential role of the immune response to HSP in cases of silent male genital tract infection and infertility. Bohring et al. (2004) have also shown that antisperm antibodies (ASA) might affect sperm motility. They found that immunofluorescence might be a valuable tool in the diagnosis of immune infertility. Immunoinfertility is indeed one of several causes of infertility in humans. Although progress on antisperm immunity and infertility has occurred during the past three decades, the nature of a real ASA is still poorly understood (Lu et al. 2008).

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In conclusion, the findings of the present study may point to a possible association between male infertility, diminished semen quality and periodontal infection in men attending fertility and IVF clinics. However, due to the small sample size and less than ideal statistical methods used, our findings need to be interpreted with caution within the context of our study.

It can be postulated that the mechanisms described above might support the hypothesis that periodontal disease is a possible risk factor for male infertility. However, it might also be plausible that periodontal disease is merely a risk indicator for infertility, arising from common exposures such as environmental, nutritional, stress-related, behaviour-oriented or genetic. These could be monitored in a future investigation.

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Clinical Relevance

Scientific rationale for the study: An association has been found between periodontal diseases and preterm low birth weight, coronary heart disease and cerebral infarction. Anaerobic gram-negative bacterial plaque that forms sub-gingivally is the direct causative factor for the development of gingivitis and periodontitis and its possible association with male infertility has not yet been investigated.
Principal findings: Familial infertility was found to be associated with patients having at least one WHO parameter contributing to infertility. CAL were significantly associated with sperm sub-motility.
Practical implications: These findings may point to an association between male infertility, diminished semen quality and periodontal disease.

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