The Effect of *In Vitro* Fertilization on Gingival Inflammation According to Women's Periodontal Status: Clinical Data

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Abstract

Objectives: To study whether *in vitro* fertilization (IVF) treatment has any effect on women's pre-existing periodontal status and, if pre-existing women's periodontal status has any impact on IVF outcomes, such as superovulation for multiple follicles maturation, oocyte retrieval and embryo transfer, as well as on pregnancy and its outcomes. **Methods:** Sixty women aged 29 to 41 years were recruited in the study. Gingival inflammation (simplified gingival index, GI-S), plaque levels (plaque control record index, PCR), bleeding on probing (BOP) and probing depth (PD), were recorded for all participants before and after IVF. Blood tests were performed prior to IVF. **Results:** A statistically significant increase in GI-S after IVF was observed in all women (31.9 ± 18.7% to 61.7 ± 23.5%), and was higher in women with gingivitis (37.1 ± 5.7% to 77.6 ± 6.7%). Women with periodontitis demonstrated a statistically significant increase in BOP (67.7 ± 6.6% to 89.5 ± 7.1%), and in the sum of probing pocket depths (from 243.8 ± 56.2 mm to 250.5 ± 58.3 mm). A trend for negative correlation between the number of follicles and transferred embryos and the gingival index, before and after IVF respectively, was recorded in all women. There was a similar trend with bleeding on probing after IVF in women with periodontitis. **Conclusions:** Periodontal clinical parameters worsened in women undergoing IVF treatment. On the other hand, a poor pre-existing periodontal status seems to be associated with poorer outcomes of IVF treatment.

Key words: In vitro fertilization; clinical periodontal status; number of follicles and transferred embryos; attainment and evolution of pregnancy

Introduction

The Word Health Organization (WHO) estimates that approximately 8-10% of all couples worldwide have a fertility problem, defined as failure of a couple to conceive after one year of frequent unprotected intercourse (Rosene-Montella *et al.*, 2000).

In vitro fertilization (IVF) refers to a procedure designed to overcome infertility and produce a pregnancy. Ovaries are stimulated using a combination of fertility medications, either to increase ovulation induction (Lin *et al.*, 2002) or to control the timing of ovulation so that the oocyte can be retrieved and used for IVF treatment (Mantzavinos *et al.*, 1997). The overall success of IVF treatment and the pregnancy rate depends on a number of factors, such as the woman's age, infertility diagnosis, and past reproductive obstetrical history (Baker *et al.*, 2010).

Periodontal disease is a common condition affecting the tissues that compromise the dental supporting structure: gingiva, cementum, periodontal ligament, and alveolar bone (Williams, 1990). Multiple studies suggest an association between pre-existing periodontal disease and adverse pregnancy outcomes such as preterm birth, low birth weight, miscarriage or early pregnancy loss, and pre-eclampsia (Gibbs, 2001; Offenbacher *et al.*, 2001; Xiong *et al.*, 2006; Agueda *et al.*, 2007). Furthermore, a recent study showed that periodontal disease could be a potential modifiable risk factor negatively affecting a non-Caucasian's woman chance of conceiving (Hart *et al.*, 2012). On the other hand, sex steroid hormones have an adverse effect on periodontal disease (Preshaw, 2013). Thus, oral contraceptives worsen the inflammation of periodontal disease (Knight and Wade, 1974; Brusca *et al.*, 2010). However, to date, little is known about the effect of exogenously administered sex hormones, such as those used in IVF procedures, on gingival inflammation.

Haytac *et al.* (2004) studied the effect of ovarian induction on the gingival tissues of women undergoing infertility treatment. The authors concluded that

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ovulation induction, by clomiphene citrate, follicle stimulating hormone (FSH) or human menopausal gonadotropin, exacerbated gingival inflammation, bleeding, and gingival crevicular fluid and that the duration of the usage of these drugs was strongly associated with the severity of gingival inflammation.
To our knowledge, no data exist on the possible effect of a women's pre-existing periodontal status on the outcome of infertility treatment with IVF.

The purpose of the present study was to study the effect of IVF treatment on women's pre-existing periodontal status, as well as to study the effect of pre-existing women's periodontal status on IVF outcomes within an IVF cycle, such as ovarian super-stimulation for development of multiple follicles, oocyte retrieval, and embryo transfer. We also studied the effect of pre-existing women's periodontal status on pregnancy rates and outcomes.

Materials and methods

Sixty women, spouses in infertile couples, aged 29 to 41 years (average age 35.5 ± 4.5 years) who attended the IVF private clinic "Embryoland" in Athens-Greece, were recruited in the study. Of 118 women who were initially examined, only 60 completed the IVF treatment and enrolled in our study from October 2009 through July 2010.

Patient inclusion criteria were: women in good health with no chronic illness, no previous hormone intake, no participation in an IVF program during the past six months, presence of at least 20 teeth and all teeth groups, absence of extensive tooth restorations (involving no more than ⅔ of the crown). Exclusion criteria were: smoking, the use of any antibiotic, antiseptic or antimicrobic treatment or any systemic medication that has an impact on periodontal tissues or induces gingival overgrowth, or a history of periodontal treatment during the past 12 months.

Subjects were informed of the purpose of this clinical study as well as the procedure to be followed, and signed a consent form. The study protocol was conducted in accordance to the Helsinki Declaration of 1975, as revised in 2008 and was approved by the Ethics and Research Committee of the Dental School, University of Athens, Greece.

The protocol in which they would participate (either the long or short), was decided according to results of the blood tests, past reproductive obstetrical history and the specific cause of infertility. Other factors affecting their enrollment in either protocol were control of pituitary and ovarian function, minimizing possible severe complications such as ovarian hyperstimulation. Treatment consisted of administering gonadotropin-releasing hormone agonists (GnRH-a, brand names Daronda 10/L, 20/L, 10/S) (long protocol) to prevent a spontaneous luteinizing hormone (LH) surge, leading to ovulation prior to oocyte retrieval. For the short protocol, gonadotropins (Gn; recombinant FSH or menopausal hMG) were administered at doses and frequency according to individual ovarian response. All women who took part in the present study received human chorionic gonadropin (hCG) and progesterone (utrogestan) per os or in vaginal suppository form, in order to simulate the endogenous LH surge and to bring about oocyte maturation (Goldberg *et al.*, 2007).

All women prior to IVF underwent blood tests and a clinical periodontal examination, which was repeated after IVF and on the day of the pregnancy test. This examination included a full-mouth simplified gingival index (GI-S), plaque control record index (PCR) according to Lindhe (1981) and O'Leary *et al.* (1972), probing depth (PD; Glavind and Loe, 1967), and bleeding on probing (BOP). Measurements for all teeth except third molars were recorded using a manual periodontal probe (PCPUNK-15, Hu-Friedy, Leiman, Germany) at four sites for gingival and plaque indices and in six sites for probing depth and bleeding on probing. The clinical examination was performed for all women by the same trained periodontist (A.P.) Intra-examiner reliability tests for reproducibility of measurements were performed prior to the study (k> 0.85).

Gingivitis was defined as > 10% of surfaces bleeding after light mechanical stimulation by the periodontal probe with no evidence of periodontal pockets (Becerik *et al.*, 2010), while adult periodontitis was defined when two or more sites exhibited probing depth ⩾ 4 mm (Kawamoto *et al.*, 2010). According to the above criteria, 20 women presented with a healthy periodontium (average age 33.2 ± 3.2 years), 19 women had gingivitis (average age 33.8 ± 3.6 years) and 21 women had periodontitis (average age 39.3 ± 3.7 years).

In all women, as well as in women grouped according to their pre-existing periodontal status, the following parameters and outcomes of IVF treatment were examined: i) administration of GnRH-a or Gn; ii) the result of the ovarian stimulation determined by estradiol levels, the number of follicles or transferred embryos. Furthermore, the overall success of the IVF treatment, defined as attainment and the evolution of pregnancy, was also examined in the same groups.

Statistical analysis

The Kruskal-Wallis or Fisher's tests were used to compare the median or mean values concerning characteristics and outcomes related to the IVF treatment. Student's t-test with Bonferroni correction or one-way ANOVA were used respectively to compare two or more mean values of indices prior to and after IVF. Correlation of the mean value of indices with the number of follicles and embryos or with the mean value of estradiol was performed using the non-parametric Spearman rank order correlation coefficient. Additionally, the comparison of indices according attainment or evolution of pregnancy was

Table 1. Parameters, outcomes (mean and median values) and success of IVF treatment: i) in all subjects (60 women); ii) when women were grouped according to their pre-existing periodontal status.

IVF parameters	All women	Women with healthy periodontium	Women with gingivitis	Women with periodontitis	Statistical analysis
Gonadotropin releasing hormone (GnRH-a)					
Daronda 20/L	47• (78.3)••	15• (75.0)••	17• (89.5)••	15• (71.4)••	0.29[a]
Daronda 10/L7	7• (11.7)••	4• (20.0)••	0•	3• (14.3)••	
Daronda 10/S6	6• (10.0)••	1• (5.0)••	2• (10.5)••	3• (14.3)••	
Gonadotropins (Gn)					
r-FSH HMG	31 (51.7) 29 (48.3)	10 (50.0) 10 (50.0)	12 (63.2) 7 (36.8)	9 (42.9) 12 (57.1)	0.43[b]

IVF outcomes	All women	Women with healthy periodontium	Women with gingivitis	Women with periodontitis	Statistical analysis
Number of follicles*	6 (4-10)	6 (5-10)	7 (4-9)	5 (2-9)	0.25[b]
Number of embryos*	3 (2-3)	3 (3-3)	3 (1-3)	2 (2-3)	0.22[b]
Mean estradiol levels**(pg/ml)*	2,024.5	2,427.5	1,956	1,892	0.25[b]
Attainment of pregnancy					
No	39• (65.0)••	11• (55.0)••	12• (63.2)••	16• (76.2)••	0.36[b]
Yes (+ biochemical)	21• (35.0)••	9• (45.0)••	7• (36.8)••	5• (23.8)••	
Evolution of pregnancy					
Biochemical	3 (5.0)	0	1 (5.3)	3 (14.3)	0.09[a]
Rejection 1st trimester	6 (10.0)	2 (10.0)	3 (15.8)	0	
Birth of one or two infants	12 (20.0)	7 (35.0)	3 (15.8)	2 (9.5)	

*Median value, **mean value; [a]Fisher's exact test, [b]Kruskal-Wallis test; •number of women, ••percent of women

performed using Student's *t*-test or Mann Whitney sign-ranked test, and one-way ANOVA or Kruskal-Wallis test, respectively. Statistical analysis was conducted using the SPSS v.18 software package (*PASW* Statistics). The level of statistical significance of all comparisons was set at 5% ($p \leqslant 0.05$).

Results

No statistically significant differences were found among the 3 groups of women, when the IVF

Table 2. Mean values and standard deviations (SD) of clinical indices prior to and after an *in vitro* fertilization (IVF) treatment in all women, and when they were grouped according to their pre-existing periodontal status.

Clinical indices	All subjects (60 women) x ± SD*	Healthy periodontium (20 women) x ± SD*	Gingivitis (19 women) x ± SD*	Adult periodontitis (21 women) x ± SD*
Simplified gingival index (GI-S %)				
Prior to IVF	31.9 ± 18.7[b]	7.9 ± 1.2[a, b]	37.1 ± 5.7[a, b]	50.2 ± 7.1[a, b]
After IVF	61.7 ± 23.5[b]	29.8 ± 4.4[a, b]	77.6 ± 6.7[a, b]	77.6 ± 7.4[a, b]
Plaque control record (PCR %)				
Prior to IVF	44.3 ± 21.1	16.2 ± 4.1[a]	57.6 ± 8.4[a]	58.9 ± 6.9[a]
After IVF	48.5 ± 21.0	20.7 ± 5.8[a]	61.9 ± 8.0[a]	62.8 ± 7.4[a]
Bleeding on probing (BOP %)				
Prior to IVF	--	--	--	67.7 ± 6.6[b]
After IVF	--	--	--	89.5 ± 7.1[b]
Sum of pocket depths (mm)				
Prior to IVF	243.8 ± 56.2[b]	–	--	243.8 ± 56.2[b]
After IVF	250.5 ± 58.3[b]	--	--	250.5 ± 58.3[b]

*Mean ± standard deviation; [a]one-way analysis of variance (ANOVA); $p < 0.001$; [b]Student's *t*-test with Bonferroni correction (paired comparison prior to and after IVF), $p < 0.001$.

Table 3. Spearman correlation coefficient values among parameters (clinical periodontal indices, number of follicles and embryos, estradiol) in all women or when they were grouped according to their pre-existing periodontal status, prior to and after an *in vitro* fertilization (IVF) treatment.

Clinical indices		Number of follicles r (*p*)*	Number of embryos r (*p*)*	Mean value of estradiol r (*p*)*
All subjects (60 women)				
Simplified gingival index	Prior to IVF	**-0.26 (0.04)**	-0.24 (0.06)	-0.19 (0.14)
	After IVF	-0.18 (0.16)	**-0.28 (0.03)**	-0.13 (0.33)
Healthy periodontium (20 women)				
Simplified gingival index	Prior to IVF	-0.13 (0.60)	-0.20 (0.40)	-0.14 (0.55)
	After IVF	-0.23 (0.32)	-0.17 (0.48)	0.07 (0.76)
Gingivitis (19 women)				
Simplified gingival index	Prior to IVF	-0.07 (0.77)	-0.22 (0.36)	-0.19 (0.42)
	After IVF	-0.12 (0.62)	-0.44 (0.06)	0.08 (0.75)
Adult periodontitis (21 women)				
Simplified gingival index	Prior to IVF	-0.31 (0.18)	-0.24 (0.30)	0.04 (0.88)
	After IVF	-0.05 (0.83)	0.007 (0.98)	0.12 (0.59)
Bleeding on probing	Prior to IVF	0.17 (0.46)	-0.02 (0.94)	-0.24 (0.30)
	After IVF	**-0.45 (0.04)**	**-0.50 (0.02)**	0.25 (0.27)
Sum of pocket depths (mm)	Prior to IVF	-0.15 (0.52)	-0.015 (0.95)	-0.12 (0.60)
	After IVF	-0.19 (0.41)	-0.043 (0.85)	-0.11 (0.62)

*Spearman rank order correlation coefficient (r), level of statistical significance (*p*). Bold indicates statistical significance.

Table 4. Comparison between mean values of clinical periodontal indices and attainment or evolution of pregnancy, in all women and when they were grouped according to their pre-existing periodontal status, prior to and after an *in vitro* fertilization (IVF) treatment.

Clinical indices		Attainment of pregnancy (*p**)	Evolution of pregnancy (*p***)
All subjects (60 women)			
Simplified gingival index	Prior to IVF	0.36	0.17
	After IVF	0.48	0.38
Healthy periodontium (20 women)			
Simplified gingival index	Prior to IVF	0.06	0.16
	After IVF	0.76	0.90
Gingivitis (19 women)			
Simplified gingival index	Prior to IVF	0.58	0.33
	After IVF	0.90	0.97
Adult periodontitis (21 women)			
Simplified gingival index	Prior to IVF	0.93	0.85
	After IVF	0.56	0.76
Bleeding on probing	Prior to IVF	0.19	0.08
	After IVF	0.32	0.22
Sum of pocket depths	Prior to IVF	0.17	0.43
	After IVF	0.22	0.50

*Level of significance after Student's *t*-test or Mann Whitney signed rank test with Bonferroni correction; **level of significance after one-way ANOVA or Kruskal-Wallis test

parameters and outcomes were examined: i) administration of GnRH-a or Gn ($p = 0.43$ and $p = 0.29$, respectively); ii) the result of the ovarian stimulation, determined by estradiol levels ($p = 0.25$), the number of follicles ($p = 0.25$) or transferred embryos ($p = 0.22$). Similarly, the overall success of IVF with respect to attainment and the evolution of pregnancy ($p = 0.36$ and $p = 0.09$ respectively) were not significantly different (*Table 1*).

After IVF treatment, a statistically significant increase in GI-S index was found in all women (Student's *t*-test, $p < 0.001$). Significant changes were also found after the treatment when women were divided into three groups: women with gingivitis, periodontitis, or healthy periodontium. In addition, bleeding on probing and pocket depth increased significantly in the periodontitis group ($p < 0.001$), after IVF. There were no significant changes in PCR index. The mean values of all these indices are shown in *Table 2*.

Relationships between periodontal and IVF parameters are shown in *Table 3*. A negative correlation was found for all women between GI-S index and the number of follicles prior to IVF ($r = -0.26$, $p = 0.04$) and between GI-S index and the number of embryos after IVF ($r = -0.28$, $p = 0.03$). A negative correlation was also found in women with periodontitis between bleeding on probing and both follicle count ($r = -0.45$, $p = 0.04$) and embryo number after IVF ($r = -0.50$, $p = 0.02$).

Finally, statistically significant differences were not observed in attainment and evolution of pregnancy overall in women according to their periodontal status in relation to their mean values of GI-S index before and after IVF (*Table 4*).

Discussion

To our knowledge, this study is the first to assess possible correlations between pre-existing periodontal status and the outcome of IVF treatment, as well as possible correlations between the pre-existing periodontal status and the overall success of the IVF, including pregnancy attainment and evolution.

Pre-existing periodontal status had no effect on measured parameters and outcomes of the IVF protocols such as type and dose of gonadotropin medication (Gn or GnRH-a drugs), estradiol levels, number of follicles and transferred embryos. In

addition, it had no effect on either attainment or evolution of pregnancy. However, our results show a statistically significant increase of gingival inflammation levels, without a corresponding change in plaque levels. This increase was observed in all women undergoing IVF treatment and was even more marked in women with gingivitis.

We also found negative correlations between the number of received follicles and simplified gingival index before IVF as well as between the number of transferred embryos and simplified gingival index after IVF. Additionally, in the periodontitis group, the levels of bleeding on probing and the sum of probing pocket depth increased after IVF. In the same group, a negative correlation was found between bleeding on probing and the number of received follicles or transferred embryos after IVF.

The limitations of our study include a small number of women in each group, and that the periodontitis group consisted of subjects with only mild or moderate chronic adult periodontitis. Both of these limitations could account for the fact that no statistically significant differences were found when the IVF parameters and outcomes, as well as the success of pregnancy attainment and evolution, were compared in women with different periodontal status. This is further supported by the fact that significant correlations were indeed found between the simplified gingival index, a measure of gingival inflammation, and the different parameters and outcomes of IVF.

The only study in the literature that examines the effect of gonadotropin medications used for ovulation induction on the gingival tissues is that of Haytac *et al.* in 2004. The authors studied the effect of three different drug protocols on the gingival tissues of women with ovulatory disorders who were undergoing infertility treatment. In this study the medications used (as monotherapy or in combination) were: clomiphene citrate, FSH, and human menopausal gonadotropin. The results showed that ovulation induction exacerbated gingival inflammation, bleeding and gingival crevicular fluid. The duration of the usage of the drugs was strongly associated with the severity of gingival inflammation. These findings are in accordance with our results. Indeed, we also found an increase of gingival inflammation and bleeding in all women who took part in the IVF treatment, as well as when women were examined in three groups according to their pre-existing periodontal status. Our study differs from that of Haytac *et al.* (2004) in type, dose and duration of gonadotropin medication.

The results of our study are also in agreement with various cross-sectional and longitudinal studies. In these studies the effect of sex steroid hormones on the periodontium was evaluated, and a significant increase of gingival inflammation resulting from the changes in vascular permeability was found (Miyagi *et al.*, 1993; Mariotti, 1994; Mascarenhas *et al.*, 2003). Furthermore, studies show that oral contraceptives, which are widely used, also have a significant influence on the periodontium, such as an increased inflammation and gingival crevicular fluid (Knight and Wade, 1974; Brusca *et al.*, 2010). These drugs, when used for long periods of time, have been associated with progression of periodontal disease, e.g., higher gingival index scores and increased loss of clinical attachment (Pankhurst *et al.,* 1981; Mascarenhas *et al.*, 2003; Mullally *et al.,* 2007).

In conclusion, our results show that IVF treatment may have a negative effect on the pre-existing periodontal status of women, as indicated by the significant increase in the gingival index. Moreover, pre-existing periodontal inflammation, as measured by the gingival index and bleeding on probing, is negatively associated with IVF outcomes, including the number of mature follicles and embryo transfer, especially in women with periodontitis. Further studies with more women are needed to determine the effect of pre-existing periodontal status on the outcome of IVF treatment.

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