Volume 09, Issue 02 (March-April 2026), PP 17-25 **www.ijmsdr.org**
ISSN: 2581-902X

International Journal of Medical Science and Dental Research

Reproductive hormones and semen quality among fertile adults with and without periodontitis: A cross-sectional study

Dr. Rim M. Ghanem[1], Dr. Mazen A. Ghanem[2*], Dr. Manal A. Safan[3], Dr. Ahmed A. Ali[4], Dr. Ahmed M. Ghanem[5], Dr. Dalia M. Ghalwash[6]

[1-]Demonstrator, Department of Oral Medicine and Periodontology, Faculty of Dentistry, The British University in Egypt (BUE), Cairo, Egypt.
[2-]Professor, Department of Surgery, College of Medicine, Al-Ayen Iraqi University, An Nasiriyah, Iraq; Department of Urology, Menoufia University, Shebin El-Kom, Egypt.
[3-] Professor, Department of Medical Biochemistry and Molecular Biology, Menoufia University, Shebin El-Kom, Egypt.
[4-]Assistant Lecturer, Department of Surgery, College of Medicine, Al-Ayen Iraqi University, An Nasiriyah, Iraq.
[5-] Medical Intern, Kasr Al-Ainy Faculty of Medicine, Cairo University, Cairo, Egypt.
[6-]Professor, Department of Oral Medicine and Periodontology, Faculty of Dentistry, The British University in Egypt (BUE), Cairo, Egypt.

ABSTRACT: Aim: This study aimed to investigate the relationship between reproductive hormone levels and semen parameters in relation to periodontal disease in fertile men.
Methods: Data from 95 fertile participants over 30 years old, whose partners were currently pregnant, who had periodontitis on periodontal examination, and who had detailed available reproductive hormone levels and semen quality, were included in our analysis. Correlations between the presence and absence of periodontitis and levels of both reproductive hormones (total testosterone (T), FSH, and inhibin-B) and semen quality in fertile men were determined and analysed.
Results: Fertile men with periodontitis had significantly lower sperm concentration and normal sperm morphology than men without periodontitis (p=0.013; 0.006, respectively). FSH levels were slightly higher, while inhibin-B levels were slightly lower in fertile men with periodontitis, without a significant difference. Interestingly, T levels were also significantly lower in fertile men with periodontitis than in those without periodontitis. Despite these differences, there was no difference between the groups in relation to the time to achieve pregnancy or the nature of pregnancy.

Conclusions: We conclude that even in fertile men, periodontitis is associated with some degree of testicular hypofunction. These findings are consistent with the existence of an association of periodontitis with reproductive hormone levels, especially T, and semen parameters in fertile men.

Keywords - *Fertile Men, Inhibin-B, Periodontitis, Semen parameters, Testosterone*

I. INTRODUCTION

Although periodontitis is a widely accepted identifiable male factor in infertile couples, the benefit of periodontitis treatment in improving pregnancy and live birth rates remains uncertain [1-3]. While it is acknowledged that periodontitis can be present in fertile men, the majority of studies describing semen parameters and reproductive hormone profiles in men with periodontitis have been in men referred for fertility evaluation [4-7].

Moreover, several published reports confirmed the positive impact of periodontitis on the serum levels of male sex hormones (total testosterone (T), SHBG, free T concentrations, DHEAS, and the free androgen index) and seminal parameters [8-11].

However, to the best of our knowledge, no clinical studies in periodontitis men of known fertility have been done, and most were done only in infertile men. The purpose of this study is to examine the association between periodontitis and the outcome of fertility, in terms of semen parameters and reproductive hormonal values, in fertile patients.

II. PATIENTS AND METHODS

2.1. Study design

The medical and dental records of patients referred for a dental examination before the initiation of periodontitis therapy served as the basis for this retrospective cohort analysis. Both the Andrology Department at Menoufia University Hospital in Egypt and the Oral Medicine and Periodontology Department at the British Hospital in Cairo, Egypt, conducted follow-up studies.

2.2. Sample size

The formula n=2(Za + Zb)2 [s]2/d2 was used to determine the sample size. Taking into account the mean and standard deviation from the literature, Zb has a value of 0.82 and Za is the z variate of alpha error, or a constant with a value of 1.96. It was calculated that 38 people per group were required to reach 95% statistical power of the study based on these data and an alpha of 0.05.

2.3. Study participants

Four hundred and twenty-two (422) men enrolled and were retrospectively analysed in the study. Women pregnant with unassisted pregnancies attending prenatal clinics were prospectively recruited between March 2019 and June 2025. For this analysis we excluded 121 men (30%) who declined a physical exam; 301 (71%) fertile men were eligible for the study, but 85 (28%) men who reported a prior history of treated periodontitis and 26 (9%) men who declined to give a hormonal and semen sample, giving a total of 188 men in the final analysis cohort (Figure 1). From this fertile cohort group, ninety-five (95) patients were diagnosed and underwent periodontal treatment for periodontitis. Meanwhile, the remaining ninety-three (93) fertile males without periodontal disease were included as controls. The control group had the same exclusion criteria as the case group.

2.4. Fertility assessment

The participants were surveyed using an anonymous questionnaire that included general information concerning fertility assessment (primary or secondary infertility) and a history of semen collection for assisted reproductive technology (ART). Infertility is defined as the inability to conceive after 1 year of regular, unprotected intercourse [14].

Data on spontaneous pregnancies, history of ectopic pregnancies, and abortion were collected. Participating couples completed questionnaires that asked about their demographics, lifestyle and habits, medical and reproductive histories, and employment history. Detailed information was obtained characterising the couple's fertility attempts for each month beginning with the last menstrual period (LMP) month of the study pregnancy and extending back for the previous twelve months before the LMP month.

A thorough clinical examination and a clinical periodontal examination were performed on each male partner. Every patient also had an andrological examination. Men were inspected while standing to determine whether they had a clinical varicocele or not. The testis was examined using scrotal Doppler ultrasonography. At the time of the dental examination, the dentist had no knowledge of the patient's andrological condition. For a period of 12 months, the couples were followed up with visits at 3-month intervals.

2.5. Clinical dental and periodontal examination

We recorded the existence of fillings, cavities, radices, and missing teeth. There were no X-rays taken. With the exception of the third molars, all individuals underwent full-mouth periodontal probing and charting using a manual periodontal probe (Hu-Friedy PCP2TM) and a plane mirror. When it was not possible to measure the probing depth (PD), the wisdom teeth and those radices were eliminated from periodontal charting [7]. The American Academy of Periodontology (AAP)/Centers for Disease Control (CDC) case definitions for periodontitis surveillance served as the basis for the diagnosis [13].

2.6. Inclusion criteria

Fertile patients (aged 31-46) with complaints of periodontal diseases were initially enrolled in the study. At least 16 natural teeth were required, and patients with periodontal disease were recruited as samples. Patients who had received systemic antibiotics, steroids, or nonsteroidal anti-inflammatory drugs within 30 days of study enrolment, as well as those who had received periodontal therapy within the preceding 3 months, were excluded before being included in the study. Additionally, people who would require antibiotic coverage for periodontal exams were not included in the enrollment.

2.7. Exclusion criteria

Varicocele, other urogenital conditions (undescended testes, testicular microlithiasis, hypogonadism, genital infection), erectile dysfunction drugs, genetic disorders, chronic infectious diseases, exposure to gonadotoxins, smoking, sperm antibodies, vascular diseases, obesity, endocrinological (e.g., diabetes), and internal diseases were excluded before including fertile patients. Additionally excluded were patients with azoospermia and sperm concentrations $<1\times10^6$/mL. Patients who refused physical examination and other testing were also excluded. To rule out alternative causes of infertility, such as ovulatory issues or tubal obstruction, the female's partners were also examined. The decision was made to rule out an ART pregnancy.

2.8. Laboratory analysis

Analysis of the semen was done using WHO guidelines (2010) [14]. The percentage of motile sperm, the percentage of abnormal forms, and the sperm concentration (million/mL) were assessed. Before treating periodontitis, semen parameters were evaluated. Morphology was evaluated using the WHO's 1999 strict criteria [15].

The electrochemiluminescence immunoassay (ECLIA) method (Elecys 1010, Boehringer Mannheim, Germany) was used to assess serum total testosterone (T) [16,17]. The Immulite automated analyser (Immulite, Diagnostic Products Corporation, Los Angeles, CA) was used to measure serum FSH. The enzyme immunoassay approach previously published by Lambert-Messerlian et al. [18] and Groome et al. [17] was used to measure serum inhibin-B. At the baseline examination, the levels of serum inhibin B, FSH, and total T were assessed in the control and periodontitis groups.

III. STATISTICAL ANALYSIS

To analyse the data, IBM SPSS version 24.0 (Armonk, NY) was utilized. The Kolmogorov-Smirnov test was used to determine whether continuous variables had a normal distribution. Data were presented as medians (interquartile range [IQR]) or means (with standard deviation [SD]) based on the distribution state. When the data were normally distributed, the student's t-test was used to compare differences in the intergroup means. The non-parametric Mann-Whitney U test was employed to compare intergroup median values for parameters having an abnormal distribution. Categorical variables with groups were analysed using the chi-

squared test. We used the methodology described by Chawre et al. [19] to categorise each man's semen analysis as either "Oligoasthenoteratozoospermia (OAT)" (sperm concentration <15 million sperm/mL, total motility <42%, and normal morphology (strict) <4%), "Normozoospermia" (sperm concentration ≥15 million sperm/mL and total motility ≥40%, and normal morphology (strict) ≥4%), or "intermediate" (either one or two parameters: sperm concentration <15 million/mL and/or motility <32% and/or morphology (strict) <4%) [19]. P-values less than 0.05 were considered significant

.

IV. RESULTS

4.1. General characteristics

The demographic data of fertile participants are summarised in Table 1. The median [interquartile range; IQR] age in the fertile men with and without periodontitis was 35.56 (34.25-38.23) years vs. 35.33 (34.12-36.33) years, respectively. Twenty-three (24.2%) and 72 (75.8%) patients had moderate and severe periodontitis, respectively. In addition, 77 (81.1%) were classified as having localised periodontitis, and 18 (18.9%) had generalised periodontitis. The median postoperative follow-up period was 33.67 (29.78-35.78) months (Table 1).

There was no difference in BMI and testicular volume in fertile men with or without periodontitis. 86 (45.3%) of the 188 men had partners who were currently pregnant at the time of the study. There was no difference between men with and without periodontitis in the time to conceive the pregnancy, the median time of pregnancy, or the nature of the pregnancy (p=0.228, p=0.415, and p=0.127, respectively).

4.2. Semen analysis characteristics

Semen analysis with and without periodontitis showed a significant decrease in sperm concentration (p=0.013). In addition, a significant decrease in the normal sperm morphology was observed between fertile men with and without periodontitis (p=0.006). However, progressive motility did not show any significant difference in fertile men with periodontitis compared to men without (p= 0.955). In general, when we categorised semen parameters, fertile men with periodontitis were more likely to have a significantly lower overall semen quality than men without periodontitis (p=0.001). Overall, abnormal semen parameters were detected in 71 (74.8%) of periodontitis patients. At the same time, 81.7% of men without periodontitis were more likely to have a sperm concentration ≥15 million/mL. However, the semen parameter has no significant correlation to fertility assessment (time to conceive and nature of pregnancy) (p > 0.5) (Table 2).

4.3. Sex hormone levels

Table 2 shows the comparison of hormone levels between the fertile male participants with and without periodontitis. Total testosterone (T) was significantly lower in men with periodontitis compared to men without periodontitis (p=0.032). Median T level was lower in the periodontitis group compared to the non-periodontitis group, 4.66 (4.32-6.34) vs. 5.31 (4.45-9.27) ng/mL, respectively. In contrast, FSH levels were slightly higher in men with periodontitis (p=0.653), although the differences were small and median values were well within the normal range for both groups. There was no significant difference in inhibin-B levels between the periodontitis and the non-periodontitis groups (p=0.150). Periodontitis was more prevalent in men presenting with low total T (57.9%, compared to 42.21% in the reference group). Even 87.3% of low total T was associated with increased severity of periodontitis (p=0.002). No significant changes in inhibin-B levels were observed in the periodontitis group compared to normal fertile participants (215.34 (196.85-219.34) vs. 216.65 (207.25-220.67) pg/mL^{-1}, P=0.150). However, the hormonal parameter has no significant correlation to fertility assessment (time to conceive and nature of pregnancy) (p > 0.5) (Table 2).

V. FIGURES AND TABLES

Table 1: Patient's demographic and clinical data

	Periodontitis group	Non-periodontitis	P

		group	Value
Number of patients	95	93	
Age at presentation, yrs*	35.56 (34.25-38.23)	35.33(34.12-36.33)	0.884
Follow-up, months*	33.67 (29.78-35.78)		
BMI (Kg/m^2)	24.20 (23.40-25.40)	23.90 (23.3-24.70)	0.751
Time to conceive (or Pregnancy) (months)	6.2 (4.09-11.23)	6.1 (5.09-8.23)	0.228
Median length of pregnancy (weeks)	38.58(33.89-39.38)	37.89(37.12-38.69)	0.415
Nature of pregnancy			0.127
Normal	80	84	
Abortion	10	9	
Ectopic	5	2	
Left testes volume (mL)	19.27(17.90-20.34)	19.12(15.89-20.17)	0.246
Right testes volume (mL)	19.46(18.70-22.11)	19.67(18.80-22.09)	0.180
Semen quality (%)			**0.001**
Normozoospermia	24	36	
Intermediate (all others)	49	43	
Oligoasthenoteratozoospermia (OAT)	22	16	
Severity of periodontitis			
Mild	-		
Moderate	23 (24.2%)		
Severe	72 (75.8%)		
Site of Periodontitis			
Localised	77 (81.1)		
Generalised	18 (18.9%)		

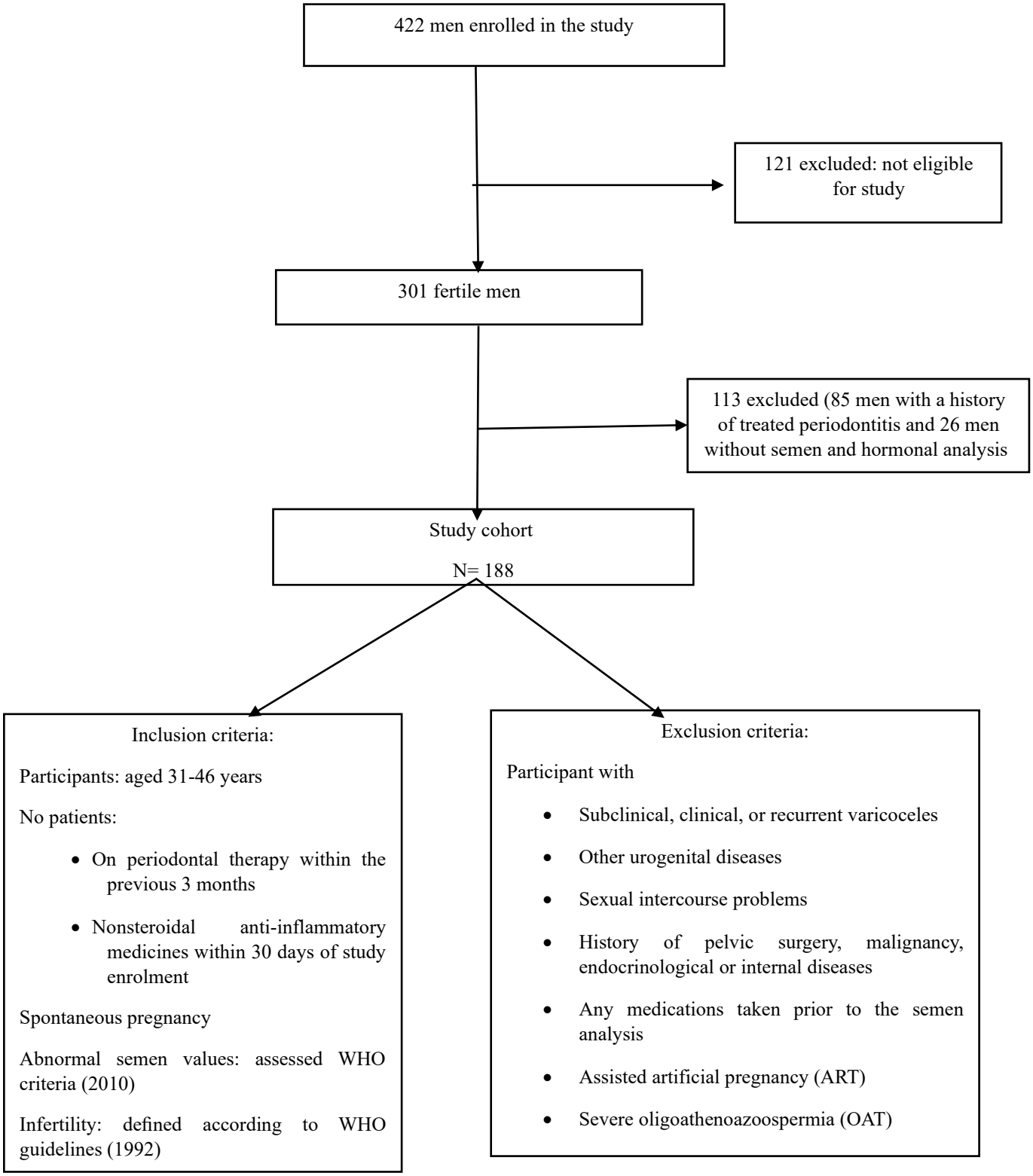
Values are presented as median (interquartile range, IQR)
P-value (Comparison between the periodontitis patient group and the non-periodontitis group)

Table 2: Changes in semen parameters and hormonal concentration of the studied fertile men with and without periodontitis

	Periodontitis patient group	Non-periodontitis Control group	±P value	*P value	°P value
Sperm concentration (million/mL)	14.25 (12.67-39.13)	**22.26 (18.22-32.34)**	0.013	**0.393**	**0.080**
Progressive sperm Motility (%)	30 (29-39)	**31 (21-42)**	**0.955**	**0.104**	**0.248**
Sperm morphology (% of normal)	**12 (3-19)**	15 (3-19)	0.006	**0.570**	**0.570**
FSH (mIU/mL)	**4.95 (3.56-6.55)**	**4.34 (3.56-5.67)**	**0.653**	**0.662**	**0.706**
Inhibin-B (pg/mL)	**215.34 (196.85-219.34)**	**216.65 (207.25-220.67)**	**0.150**	**0.174**	**0.065**
Testosterone (ng/dL)	**4.66 (4.32-6.34)**	**5.31 (4.45-9.27)**	0.032	**0.381**	**0.975**

±**P value** (Comparison between the patient group and the control group)
*__P value__ (Comparison between the participant groups and the nature of pregnancy)
°**P value** (Comparison between the participant groups and time to conceive)
Values are presented as median (interquartile range, IQR)

Fig 1. Flow chart of study design



VI- DISCUSSION

Periodontitis is a pathological condition present in over 50% of adult males aged 30 or older and in >30% of idiopathic infertile men [3,7]. Many studies have suggested a relationship between common oral diseases such as periodontitis and male reproductive health conditions, including testosterone levels and male infertility [2,4,9]. However, data on the relationship between periodontitis and reproductive hormones and the outcome of pregnancy, particularly in fertile men with periodontitis, have not been investigated, according to the literature that has been indexed. In contrast to previous research conducted in the infertile group, this study aimed to demonstrate the utilisation of total testosterone, FSH, inhibin-B levels, and semen quality as analytical tools in the assessment of periodontitis effect in fertile groups.

In the present study, we compared semen and hormone parameters in relation to the presence or absence of periodontitis in 188 fertile men participating in the study. Both sperm count and normal morphology were significantly lower in men with periodontitis, and men with periodontitis were more likely to have overall poorer semen quality using an integrated measure, although the clinical importance of this is unclear. Most men with periodontitis had semen parameters within the range seen in fertile men, and there was no difference in time to conceive pregnancy or in the history of previous pregnancy in men with or without periodontitis. Overall, they found a significant difference in the normal cell morphology between men with and without periodontitis. 81.7% of men without periodontitis were more likely to have a sperm concentration ≥ 15 million/mL.

Regarding sex hormone levels, the relationship between reproductive hormone levels in men and periodontitis has shown inconsistent findings. Controversies still exist on the impact of periodontitis on male sexual health. Some studies find no difference in T levels or differences in selected subgroups, but not all men with periodontitis, while other studies report lower levels of T in men with periodontitis. [8,9,11,20] In this current study, fertile men with periodontitis had a significantly lower testosterone level. Our findings suggest some degree of primary testicular hypofunction, at least for spermatogenesis, in men with periodontitis. In contrast, other studies found no obvious connections between sex hormones with the progression of periodontitis. [8,21].

In that study, fertile men with periodontitis had non-significantly higher FSH levels, although their mean testosterone levels were lower than those of men without periodontitis. We also observed non-significantly lower changes in serum inhibin-B in periodontitis, suggesting a decrease in Sertoli cell function and spermatogenesis as a result of the inflammatory reaction of the periodontitis. [22,23] Moreover, the concomitant increase in FSH further supports this observation and indicates restoration of the negative feedback mechanism.

Moreover, we did not find evidence that the presence of periodontitis was associated with any difficulty conceiving in our cohort. The percentage of couples with a previous pregnancy and the time to conceive were not different between fertile men with and without periodontitis. Interestingly, this study in fertile men has failed to find evidence of impaired pregnancy outcome in men with periodontitis.

However, this study had some inevitable limitations. It is important to note that the results of the included studies may have been skewed by a number of factors, and these conclusions should be regarded cautiously. Although immunoassays are regarded as a reliable technique for measuring testosterone levels, many epidemiological studies view the lack of uniformity in steroid hormone assays as a significant restriction and shortcoming [20,21,24,25,28]. Age, genetics, hormone-related medications, TSH, free T4, socioeconomic variables, alcohol, tobacco, and comorbidities, including obesity and diabetes, are some of the factors that may affect testosterone levels [26,27]. In fact, before any conclusions can be made, more carefully planned epidemiological studies are required, including those that include bone turnover markers and biomarkers of inflammation (such as fibrinogen, white blood cell count, and high-sensitivity C-reactive protein) that may affect the immune-inflammatory response [20]. The study's cross-sectional design restricted the findings on gonadal hormone levels and periodontitis to potential correlations rather than causes [29].

VI. CONCLUSIONS

In conclusion, this is the first prospective, population-based study assessing the relationships between male reproductive hormone levels and semen characteristics and fertility outcomes, particularly pregnancy outcomes, in men who are fertile but have periodontitis. In individuals with fertile periodontitis, we did not find any evidence linking poor pregnancy outcomes to serum hormone concentrations or semen quality. However,

more cohort longitudinal research and carefully planned randomised controlled trials are required, because the connection between reproductive hormone levels, semen quality, and periodontitis is still up for debate.

REFERENCES

[1] D'Aiuto F, Nibali L, Parkar M, Patel K, Suvan J, Donos N. Oxidative stress, systemic inflammation, and severe periodontitis. *J Dent Res.* 2010;89:1241–1246. doi: 10.1177/0022034510375830. Epub 2010 Aug 25.

[2] Botelho J, Machado V, Proença L, Bellini D, Chambrone L, Alcoforado G, *et al,* The impact of nonsurgical periodontal treatment on oral health-related quality of life: A systematic review and meta-analysis. *Clin. Oral Investig.* 2020; 24(2): 585-596. doi: 10.1007/s00784-019-03188-1. Epub 2020 Jan 4.

[3] Ricci E, Ciccarelli S, Agnese Mauri P, Gerli S, Favilli A, Cipriani S, *et al.* Periodontitis, female fertility and conception (Review). Biomed Rep. 2022 Sep 13;17(5):86. doi: 10.3892/br.2022.1569. eCollection 2022 Nov.

[4] Bieniek KW, Riedel HH. Bacterial foci in the teeth, oral cavity, and jaw– Secondary effects (remote action) of bacterial colonies with respect to bacteriospermia and subfertility in males. *Andrologia.* 1993; 25(3):159–62. https://doi.org/10.1111/j.1439-0272.1993.tb027 00.x.

[5] Zhu C, Qin Z, Huang H, Xiangguang LI, Feng Y. The correlation study between male infertility and chronic periodontitis. *China Modern Medicine.* 2010; *29*:8. Retrieved from http://en.cnki.com.cn/Article_en/CJFDTotal-ZGUD20 1029008.htm

[6] Pásztor N, Kárpáti K, Szöllősi J, Keresztúri M, Kozinszky Z, Gorzó I, *et al.* Association between periodontal status and idiopathic male infertility. *J Oral Sci.* 2016; 58(2):247–53. https://doi.org/10.2334/josnu sd.15-0586.

[7] Tao DY, Zhu JL, Xie CY, Kuang YP, Chai WR, Lo ECM, *et al.* Relationship between periodontal disease and male infertility: A case-control study. *Oral Dis.* 2021; Apr;27(3): 624-31. https://doi.org/10.1111/odi.13552. Epub 2020 Aug 31'

[8] Samietz S, Holtfreter B, Friedrich N, Mundt T, Hoffmann W, Völzke H, Nauck M, Kocher T, Biffar R. Prospective association of sex steroid concentrations with periodontal progression and incident tooth loss. *J Clin Periodontol.* 2016; Jan;43(1):10-8. doi: 10.1111/jcpe.12493. Epub 2016 Jan 26.PMID: 26661340.

[9] Kellesarian SV, Malmstrom H, Abduljabbar T, Vohra F, Kellesarian TV, Javed F, Romanos GE. "Low Testosterone Levels in Body Fluids Are Associated With Chronic Periodontitis". *Am J Mens Health.* 2017; Mar;11(2):443-53. doi: 10.1177/1557988316667692. Epub 2016 Sep 21.PMID: 27645514.

[10] Lecaplain B, Badran Z, Soueidan A, Prud'homme T, Gaudin A. Periodontitis, erectile dysfunction, reproductive hormones, and semen quality: A systematic review. *Andrology.* 2021 May;9(3):769-80. doi: 10.1111/andr.12961. Epub 2021 Jan 2.

[11] Su X, Jin K, Zhou X, Zhang Z, Zhang C, Li Y, Yang M, Huang X, Xu S, Wei Q, Cheng X, Yang L, Qiu S. The association between sex hormones and periodontitis among American adults: A cross-sectional study. *Front Endocrinol.* (Lausanne) 2023; Feb 14; 14:1125819. doi: 10.3389/fendo.2023.1125819. eCollection 2023.

[12] World Health Organization. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril*. 1992; 57:1289–93.

[13] Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol.* 2012;83(12): 1449–54. https:// doi.org/10.1902/jop.2012.110664.

[14] World Health Organization. WHO laboratory manual for the examination and processing of human semen, Fifth edition, 2010.

[15] Swan S, Brazil C, Drobnis E, Liu F, Kruse R, Hatch M, Redmon JB, Wang C, Overstreet JW, Study for Future Families Research Group., 2003 Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect.* 2003 Apr;111(4):414-20. doi: 10.1289/ehp.5927

[16] de Waal WJ, Vreeburg JT, Bekkering F, de Jong FH, de Muinck Keizer-Schrama SM, et al. High dose testosterone therapy for reduction of final height in constitutionally tall boys: does it influence testicular function in adulthood? *Clin Endocrinol.* 1995; 43: 87–95.

[17] Groome NP, Illingrowth PJ, Brien M. Measurement of dimeric inhibin B through the human menstrual cycle. J Clin Endocrinol Metab. 1996; 81: 1401–5.

18. Lambert-Messerlian GM, Hall JE, Sluss PM. Relatively low levels specific two-site enzyme immunoassay. *J Clin Endocrinol Metab.* 1994; 79: 45–50.

[19] Chawre S, Khatib MN, Rawekar A, Mahajan S, Jadhav R, More A. A Review of Semen Analysis: Updates From the WHO Sixth Edition Manual and Advances in Male Fertility Assessment. *Cureus.* 2024; Jun 29;16(6):e63485. doi: 10.7759/cureus.63485. eCollection 2024 Jun.

[20] Steffens JP, Wang X, Starr JR, Spolidorio LC, Van Dyke TE, Kantarci A. Associations between sex hormone levels and periodontitis in men: Results from NHANES III. *J Periodontol. 2015*; 86(10):1116–25. doi: 10.1902/jop.2015.14053

[21] Orwoll ES, Chan BK, Lambert LC, Marshall LM, Lewis C, Phipps KR. Sex steroids, periodontal health, and tooth loss in older men. *J Dent Res.* 2009; 88(8):704–8. doi:10.1177/0022034509341013.

[22] Pierik FH, Vreeburg JTM, Stijnen T, de Jong FH, Weber RFA. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab.* 1998; 83: 3110–4.

[23] Fisch H, Hyun G, Hensle TW. Testicular growth and gonadotrophin response associated with varicocele repair in adolescent males. *BJU Int.* 2003; 91: 75–8.

[24] Ankarberg-Lindgren C, Norjavaara E. Sensitive RIA measures testosterone concentrations in prepubertal and pubertal children comparable to tandem mass spectrometry. *Scand J Clin Lab Invest.* 2015 Jul;75(4):341-4. doi: 10.3109/00365513.2014.942694. Epub 2015 Feb 27.

[25]. Wilke TJ, Utley DJ. Total testosterone, free-androgen index, calculated free testosterone, and free testosterone by analog RIA compared in hirsute women and in otherwise-normal women with altered binding of sex-hormone-binding globulin. *Clin Chem.* 1987; 33(8):1372–5. doi: 10.1093/clinchem/33.8.1372.

[26]. Trost, LW & Mulhall, JP. Challenges in testosterone measurement, data interpretation, and methodological appraisal of interventional trials. *J Sex Med*. *2016;13*, 1029-1046. doi:10.1016/j.jsxm.2016.04.068.

[27] Javed, F, Bashir Ahmed, H & Romanos, GE. Association between environmental tobacco smoke and periodontal disease: A systematic review. *Environ Res. 2014; 133*, 117-122. doi:10.1016/j.envres.2014.05.008.

[28] Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab.* 1999; 84 (10):3666–72. doi: 10.1210/jcem.84.10.6079.

[29] Holtfreter B, Albandar JM, Dietrich T, Dye BA, Eaton KA, Eke PI, et al. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies: Proposed standards from the joint EU/USA periodontal epidemiology working group. *J Clin Periodontol.* 2015; 42(5):407–12. doi: 10.1111/jcpe.12392.