

1 **Effect of tobacco smoking on fertility regulating**
2 **hormones in men**

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ABSTRACT

Aims: Tobacco smoking is having a far more serious effect on reproductive health than previously thought and is responsible for many thousands of cases of impotence, miscarriages, and infertility each year. Among those experiencing difficulty with conception, a male fertility problem partly resulting from fertility hormone derangements is considered important. This study assessed the effect of cigarette smoking on fertility hormone levels in males.

Study design: This is a cross sectional study.

Place and Duration of Study: The study was conducted from January 2010 to November 2010 at the Tamale Teaching Hospital located in the Northern Region of Ghana.

Methodology: In this study, a total of 99 subjects were recruited comprising, 54 (54.5%) smokers and 45 (45.5%) non-smokers. The smokers were stratified into mild (smoke <5 sticks of cigarette/day), moderate (smoke 5–10 sticks of cigarette/day), and heavy smokers (smoke >10 sticks/day). Fertility regulating hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), testosterone (TES), oestradiol (E2II) and sex hormone binding globulins (SHBG) were assayed. Total protein (TPRO) and albumin (ALB) were also estimated.

Results: In this study, 14.1% of the smokers were mild smokers, 30.3% were moderate smokers while 10.1% were heavy smokers. FHS, LH, PRL, E2II, SHBG were not significantly different among smokers and non-smokers; however, testosterone (TES) levels (ng mL⁻¹) in smokers were significantly higher compared to non-smokers (7.33 ± 3.3 vs 5.78 ± 1.8 , $p = .01$). ALB (g/L) was significantly lower in smokers (35.91 ± 4.5 vs 41.18 ± 7.5 , $P = .04$) while GLO (g/L) was significantly higher in smokers (44.28 ± 7.7 vs 34.15 ± 8.6 , $P = .005$) compared to non-smokers.

Conclusion: This study therefore concludes that, fertility hormone levels were not significantly affected by tobacco smoking; except testosterone level, which is significantly higher in smokers compared to non-smokers.

Keywords: [cigarette smoking, tobacco, fertility hormones, smokers and nonsmokers]

1. INTRODUCTION

Environmental and/or lifestyle factors have been suggested as factors responsible for the decline in fertility. Cigarette smoking which is considered as a major public health problem [1] has the highest prevalence in young adult males in their reproductive period [2]. It is estimated that tobacco kill 10 million people a year with 70% of these deaths occurring in developing countries [1], with 1.2 million deaths resulting from second hand smoke [3]. A survey conducted in Nigeria showed that 24.4% of males and 6.7% of females smoked cigarette on daily basis [1]. In males, cigarette smoking is associated with a decreased testosterone level, destruction of sperms, decreased sperm motility, relative infertility and impotence [1].

Tobacco smoke contains numerous compounds including substances of medical significance, such as carcinogenic polycyclic aromatic hydrocarbons, irritant substances, nicotine, carbon monoxide and other gases [4]. Nicotine, the main bioactive chemical substance in tobacco, inhibits steroidogenesis in mouse Leydig cells and chronic treatment with nicotine was reported to

cause decrease in fertilization in male animals [1]. Carbon monoxide in tobacco smoke has a higher affinity for haemoglobin, thereby reducing the oxygen-carrying capacity of the blood [3, 5].

Smoking has an effect on the various metabolic and biological processes in the body including secretion of hormones. These biological processes are mediated chiefly through behavioural and pharmacological actions of nicotine and also by toxins such as thiocyanate [4]. Smoking affects pituitary, thyroid, adrenal, testicular and ovarian functions, calcium metabolism and the action of insulin. Smoking stimulates the release of several anterior and posterior pituitary hormones [4]. Cigarette smoking and intravenous nicotine infusion has been associated with increases in prolactin levels in humans [1].

Effect of tobacco smoking on sex hormones

Cigarette smoking has major effects on the reproductive potential of humans. Tobacco smoking has an anti-oestrogenic effect in women [6, 7] which is as a result of changes in hepatic oestrogen metabolism induced by smoking. Smoking has a powerful effect on the 2-hydroxylation pathway of oestradiol metabolism leading to increased production of 2-hydroxyestrogens [8]. The 2-hydroxyestrogens have minimal oestrogenic activity and are rapidly cleared from the circulation.

In circulation oestrogens bind avidly to sex hormone binding globulin (38%), loosely to albumin (60%) with the remainder being the free unbound fraction. In smokers, concentrations of SHBG are higher; therefore, lower concentrations of biologically active oestrogens are seen [9, 10]. Various studies examining the effects of smoking on serum testosterone levels have reported conflicting findings largely due to difficulties in the hormonal assays. Testosterone has a circadian rhythm with levels peaking between 0600 and 0800 h and reaching a nadir between 1800 and 2000 h. A significant proportion of the circulating total testosterone is inactive as it is tightly bound to SHBG (65–80%), whereas the biologically active fraction circulates either free (1–3%) in circulation or loosely bound to albumin (20–40%) [11].

Svartberg *et al.*, [12] found a positive association between testosterone and smoking even after adjusting for SHBG though other plasma proteins were not taken into account. The effects of smoking on testosterone levels are likely due to changes in plasma-binding capacity rather than a direct effect of nicotine on androgens. Vine, [13] reported that testosterone levels may remain unchanged, elevated or decreased while oestradiol levels were mainly found to be elevated in smokers. Briggs, [14] reported that smoking decreased plasma testosterone and attributed the reduced testosterone biosynthesis to carbon monoxide inhibition of Leydig cell microsomal hydroxylases.

Fluctuations in androgen and gonadotropin hormone levels have been documented in male smokers [15]. Experimental evidence from humans and rodents suggests that cigarette smoke or nicotine can alter hypothalamic-pituitary interrelationships, stimulating growth hormone, cortisol, vasopressin, and oxytocin release and inhibiting luteinizing hormone and prolactin release. Higher follicle stimulating hormone (FSH) levels have been found in male smokers [15]. Nicotine may suppress testicular and androgen production by altering Leydig cell function; thus, altering hormonal interrelationships necessary for successful reproduction. This study assessed the effect of cigarette smoking on fertility hormone levels.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Study Site

The study was conducted from January 2010 to November 2010 at the Tamale Teaching Hospital located in the Northern Region of Ghana. Subjects were drawn from the Tamale Metropolis. Tamale is cosmopolitan in nature with inhabitants not only hailing from the Northern origin but from other parts of Ghana and the sub-region. The climate is tropical with a rainy season from May to October and a long dry season with virtually no rainfall from October to April. Temperatures range between a maximum of 37°C in March / April and at least 18°C in December.

2.2 Study Population

Apparently healthy men between the ages of 18 and 45 years were selected at random for the study by out-reach programs. In all, a total of 99 subjects were examined.

Detailed medical history and examination was conducted by a urologist to eliminate men with any condition that could affect fertility. Demographic data, average number of cigarette sticks smoked per day, duration of smoking, the number and ages of biological children were recorded. Written and signed informed consent was obtained from all subjects who partook in the study. Ethical clearance for the study was obtained from the Research and Protocol Review Committee of the University of Ghana Medical School (UGMS).

2.2.1 Case Definition

Subjects were classified into two main groups: non-smokers (control group) and smokers (exposed group). Non-smokers were defined as subjects who have never smoked tobacco whilst smokers were defined as subjects who have smoked tobacco continuously for at least six months. The smokers were stratified into mild (smoke <5 sticks of cigarette/day), moderate (smoke 5–10 sticks of cigarette/day), and heavy smokers (smoke >10 sticks/day).

2.2.2 Inclusion Criteria

Subjects age between 18 to 45 years who were medically examined to eliminate confounders such as varicocele and testicular trauma were recruited into the study and classified as smokers or non-smokers.

2.2.3 Exclusion Criteria

All men who were above 45 years were excluded from the study to avoid age related effects on hormone secretion. Men who drink alcohol and men who have smoked tobacco for less than six months were excluded from the study.

2.3 Sample Collection and Preparation

2.3.1 Specimen collection for hormonal estimation

A total volume of 5 ml of venous blood was collected from the antecubital vein of each subject under aseptic conditions, between the hours of 6am to 9am, dispensed into a plain test tube and allowed to clot. The blood was then spun at 3000rpm for 3 minutes and the serum used for fertility hormone analysis, i.e. oestradiol (E2II), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TES), prolactin (PRL), total protein (TPRO), albumin (ALB) estimation while globulin (GLO) was calculated by subtracting ALB from TPRO. Serum samples that could not be analysed immediately were stored at -20°C until required for analysis. The hormone levels were estimated using Mini Vidas Auto Analyser (manufactured by Bio Merieux Inc, United State of America).

2.3.2 Estimation of Plasma Proteins

The BT 3000 Random Access Chemistry analyzer (Elan Diagnostics, Smithfield, CA, USA) was used for protein estimation.

3. RESULTS AND DISCUSSION

3.1 Demographic Characteristic Of the Study Population

A total of 99 subjects were recruited based on the inclusion criteria and grouped into non-smokers and smokers. In this study, there was no significant difference in the mean ages (years) of smokers (35.0 ± 5.5) as compared with non-smokers (36.6 ± 7.7) ($P = .24$) (table 2). In all, 45 (45.5%) were non-smokers while 54 (54.5%) were smokers (table 1). The smokers were further stratified into mild, moderate and heavy smokers depending on the number of sticks of cigarette smoked per day. Smokers who smoke less than five (<5) sticks of cigarette per day were classified as mild smokers, smokers who smoke between 5 to 10 sticks of cigarette per day were classified as moderate smokers while smokers who smoke more than ten (>10) sticks per day were classified as heavy smokers. Fourteen (14) smokers (14.1%) of the study population were mild

smokers, 30 smokers (30.3%) were moderate smokers while 10 smokers (10.1%) were heavy smokers.

Table 1. Basic information about the study population

| | Number Of Sticks/Day | N | Percentage (%) |
|---------------------------------|----------------------|----|----------------|
| Total number of subjects | | 99 | 100 |
| Smokers | | | |
| Mild smokers | <5 | 14 | 14.1 |
| Moderate smokers | 5-10 | 30 | 30.3 |
| Heavy smokers | >10 | 10 | 10.1 |
| Non-smokers | | 45 | 45.5 |

Table 1 shows the demographic characteristics of the study population. Data are presented as percentages.

3.2 Variation of Hormone Levels in Smokers and Non-Smokers

Table 2 shows a comparison of the levels of FSH, LH, PRL, TES and E2II in non-smokers and smokers. The mean FSH level in non-smokers (8.56 ± 8.9) was non-significantly higher than in smokers (6.64 ± 4.1) ($P = .16$). The mean LH level in non-smokers (5.64 ± 4.0) was non-significantly lower than in smokers (6.00 ± 3.2) ($P = .62$). Prolactin and oestradiol levels in non-smokers and smokers showed no significant difference, with P - values of .43 and .28 respectively. Testosterone was significantly lower in non-smokers (5.78 ± 1.8) than smokers (7.33 ± 3.3) ($P = .007$). The mean total protein level in non-smokers (76.08 ± 15.4) and smokers (79.47 ± 7.6) was not significantly different. Globulin level was significantly higher in smokers (44.28 ± 7.7) compared to non-smokers (34.15 ± 8.6) ($P = .005$). Albumin level was significantly higher in non-smoker (41.18 ± 7.5) compared with smokers (35.91 ± 4.5) ($P = .04$).

Table 2. Hormonal and protein concentrations in non-smokers and smokers

| Parameters | Total (N=98) | Non-smokers (N=45) | Smokers (N=54) | p value |
|----------------|------------------|-----------------------|-------------------|---------|
| Age (years) | 35.6 ± 6.8 | 35.0 ± 5.5 | 36.6 ± 7.7 | .24 |
| LH (mIU mL-1) | 5.83 ± 3.6 | 5.64 ± 4.0 | 6.00 ± 3.2 | .62 |
| PRL (ng mL-1) | 18.88 ± 10.1 | 18.00 ± 9.7 | 19.62 ± 10.5 | .43 |
| TES (ng mL-1) | 6.62 ± 2.8 | 5.78 ± 1.8 | 7.33 ± 3.3 | .007 |
| E2II (pg mL-1) | 25.68 ± 14.2 | 23.99 ± 13.3 | 27.11 ± 14.8 | .28 |
| TPRO (gL-1) | 77.77 ± 12.0 | 76.08 ± 15.4 | 79.47 ± 7.6 | .50 |
| ALB (gL-1) | 38.44 ± 6.7 | 41.18 ± 7.5 | 35.91 ± 4.5 | .04 |
| GLO (gL-1) | 39.42 ± 9.5 | 34.15 ± 8.6 | 44.28 ± 7.7 | .005 |

Table 2 shows unpaired t-test comparison of hormonal variations in non-smokers and smokers. FSH-follicle stimulating hormone, LH-lutenizing hormone, PRL-Prolactin, TES-Testosterone, E2II- Oestradiol, TPRO-total protein, ALB-albumin, GLO-globulins. Data are presented as mean \pm SD.

3.3 HORMONAL LEVELS IN SMOKERS

In this study, the variation of hormone levels among the various stratifications of smokers was investigated (Table 3). For each hormone, mild smokers were compared to moderate and heavy smokers and then moderate smokers were compared to heavy smokers. The variation of prolactin among mild smokers, moderate smokers and heavy

smokers was not significant ($F = .70$, $P = .50$). The levels of FSH and LH did not show any significant variation among mild, moderate and heavy smokers ($F = 0.34$, $P = 0.71$ and $F = .77$, $P = .50$ respectively). Similarly, testosterone and oestradiol did not show any significant variation among mild, moderate and heavy smokers ($F = .36$, $P = .70$ and $F = .08$, $P = .50$ respectively).

Table 3. Hormone concentrations in smokers

| PARAMETER | SMOKERS | | | F test | p value |
|-----------------------------|-------------|-------------|-------------|--------|---------|
| | Mild | Moderate | Heavy | | |
| FSH (mIU mL ⁻¹) | 5.8 ± 3.6 | 6.9 ± 4.8 | 7.1 ± 2.1 | .34 | .71 |
| LH (mIU mL ⁻¹) | 6.1 ± 3.4 | 5.6 ± 2.9 | 7.1 ± 4.0 | .77 | .47 |
| PRL (ng mL ⁻¹) | 17.1 ± 6.4 | 19.8 ± 12.8 | 22.3 ± 6.4 | .70 | .50 |
| TES (ng mL ⁻¹) | 7.7 ± 2.2 | 7.5 ± 3.7 | 6.5 ± 3.5 | .36 | .70 |
| E2II (pg mL ⁻¹) | 28.0 ± 12.5 | 27.3 ± 15.1 | 25.5 ± 17.9 | .08 | .92 |

Data are presented as mean ± SD, Table 3 shows One-way ANOVA of hormone concentrations in smokers stratified by number of sticks smoked per day. FSH-follicle stimulating hormone; LH-lutenizing hormone; PRL-Prolactin; TES-Testosterone; E2II-Oestradiol

1 DISCUSSION

2 Saadat [16] and Pasqualotto, Sobreiro [17] reported that there was no significant
3 change in FSH and LH levels among smokers and non-smokers. In this study, the
4 levels of FSH and LH were not significantly different in smokers and non-smokers.
5 Mendelson *et al.*, [18] and Kirschbaum *et al.*, [19] reported that cigarette smoking
6 resulted in higher prolactin levels. On the contrary, the results of this study found no
7 significant change in prolactin levels in smokers compared with non-smokers.
8 Furthermore, there was no significant difference in oestradiol levels in smokers and
9 non-smokers.

10 Svartberg *et al.*, [12], English *et al.*, [20] and Field *et al.*, [21] reported higher levels
11 of total testosterone in smokers compared with non-smokers. The results of this
12 study showed significantly higher levels of testosterone in smokers compared to
13 non-smokers. Dunn *et al.*, [11] reported that 65-80% of the circulating total
14 testosterone is inactive and tightly bound to SHBG, whereas the biologically active
15 fraction circulates either freely (1-3%) or loosely bound (20-35%) to albumin. Bio-
16 available testosterone is the free and the albumin-bound testosterone. Levels of
17 total testosterone can, therefore, be directly affected by changes in levels of SHBG
18 and other plasma proteins. English *et al.*, [20] demonstrated that the increase in
19 total testosterone observed in smokers was due to the raised SHBG levels and
20 further reported that SHBG levels and not testosterone correlated with serum
21 nicotine levels. English *et al.*, [20] explained that the effects of smoking on
22 testosterone levels were due to changes in SHBG-binding capacity rather than a
23 direct effect of nicotine on androgens.

24 On the contrary, Briggs *et al.*, [22] reported decreases in plasma testosterone
25 concentration in cigarette smokers. Observations document by Briggs *et al.*, [22]
26 could be attributed to the testosterone estimation method used. Briggs *et al.*, [22]
27 extracted testosterone from heparinized plasma using methylene chloride, purified
28 using thin layer chromatography, and estimated testosterone by competitive protein
29 binding assay [23], while our study estimated total testosterone by enzyme
30 immunoassay. Other studies such as Vine *et al.*, [24] measured cotinine and
31 nicotine instead of the number of cigarette sticks smoked a day.

32 This study measured total testosterone, which includes, the SHBG bound
33 testosterone, albumin bound and free testosterone, therefore changes in plasma
34 proteins and SHBG may not directly affect the levels of testosterone in both cohorts.
35 In a meta-study conducted by Zhao, Leung [25], it was reported that, cotinine which
36 is a metabolite of nicotine inhibits testosterone degradation. Cigarette Smoking
37 increases plasma testosterone concentration because nicotine and its metabolites
38 share a metabolic pathway with androgens, and so cigarette smoking competitively
39 inhibits testosterone degradation by the body, leading to the accumulation of
40 testosterone in the plasma of smokers [25]. Testosterone, cotinine and *trans*-3'-
41 hydroxycotinine (3HC) are all inactivated by conjugation (i.e. glucuronidation) from
42 the uridine 5'-diphospho (UDP)-glucuronosyltransferase (UGT) superfamily [26], the
43 same UGT enzyme used for glucuronidation of both nicotine metabolites and
44 androgens. The UGT2B10 enzyme for nicotine and cotinine, the UGT2B17 enzyme

45 for 3HC [27], and the UGT2B7, UGT2B15 and UGT2B17 enzymes for androgens
46 [26, 27].

47 In this study, serum albumin concentration was found to be significantly lower in
48 smokers compared with non-smokers. Roohi and Mehjabeen [28] and Hunter *et al.*,
49 [29] in separate studies reported that low albumin levels correlated with smoking.
50 This is because, cigarette smoking is associated with increased oxidative stress
51 [30]. Albumin also has antioxidant properties, by binding copper ions and
52 scavenging hypochloride (HOCl) present in cigarette. The scavenging of HOCl by
53 albumin may well be due to the rapid reaction with -SH groups on albumin, and
54 oxidized albumin may be cleared rapidly from the circulation and degraded [31, 32].
55 The significantly lower albumin concentration observed in smokers when compared
56 with non-smokers gives an indication of a lower bio-available testosterone
57 concentration in smokers.

58 A significantly higher total testosterone concentration was observed in the smokers
59 when compared with non-smokers. In this study, serum globulin showed a
60 significantly high concentration in the smokers compared with non-smokers,
61 suggesting that a greater percentage of testosterone is inactive and tightly bound to
62 globulins. The higher serum globulin observed in smokers in this study could be a
63 possible reason for the observed increase in total testosterone in smokers.

64 The variation of follicle stimulating hormone, luteinising hormone, prolactin,
65 testosterone and oestradiol among mild smokers, moderate smokers and heavy
66 smokers was not significant; suggesting that in this study, there was no dose
67 dependent effect of tobacco smoking on hormone levels.

68 **4. CONCLUSION**

69 **Conclusion**

70 In this study, it was observed that tobacco smoking does not have any significant effect on
71 fertility regulating hormones, except testosterone which is higher in smokers.

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73 **Limitations**

74 At the time of conducting this study, there was difficulty in getting facility to estimate serum
75 sex hormone binding globulins and nicotine. Changes in the levels of sex hormone binding
76 globulin are known to affect testosterone levels through decreases in bio-available
77 testosterone. Furthermore, serum nicotine would have been a better measure of the dose
78 effect of tobacco smoking than the number of sticks smoked per day.

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80 **Recommendations**

81 Tobacco consumption is a subjective measure due to the diversity in nicotine concentration
82 within different cigarette brands and the actual amount of smoke inhaled according to
83 personal habits. This study recommends the measurement of serum nicotine instead of the
84 number of sticks smoked per day. This study also recommends the measurement of serum
85 sex hormone binding globulins (SHBG).

86 **Statistical analysis**

87 Comparisons between the three groups were performed using one-way analysis of variance
88 (ANOVA) and unpaired *t*-test was used for comparison between two groups. All hypothesis
89 testing was two-tailed with a significance level of 0.05. All statistical analysis were performed

90 with GraphPad (www.graphpad.com) version 5.00 statistical software (GraphPad Software,
91 San Diego, CA).

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96

97 Competing Interests

98 Authors have declared that no competing interests exist.

99

100 Authors' Contributions

101 This study was designed and supervised by SBB and KOD. SBB and LQ drafted the
102 manuscript. PPD contributed to the draft of the manuscript. SBB and BG participated in the
103 recruitment and sampling of study subjects. MB and YA made financial contributions towards
104 the study and were involved in the laboratory analysis of the samples. L.Q. and KOD made
105 contributions to the study design and also helped draft the manuscript. BG supervised and
106 made intellectual contributions to the manuscript. M.B. participated in subject recruitment.
107 PPD, B.M. and L.Q was involved with the statistical analysis. All authors read and approved
108 the final manuscript.

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110 Consent

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115 ETHICAL APPROVAL

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186 DEFINITIONS

187 Non-smokers were defined as subjects who have never smoked tobacco (control group), whilst smokers were
188 defined as subjects who have smoked tobacco continuously for at least six months (exposed group).

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ABBREVIATIONS, ACRONYMS,

TES, testosterone; E2, estradiol; FSH, follicle stimulating hormone; GSS, LH, luteinizing hormone; PROL, prolactin; SHBG, sex hormone binding globulin; ALB, albumin; GLO, Globulin, TPRO, total protein.

APPENDIX