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Effect of Biomass Smoke on Female Reproductive Hormones among Caterers in NNEWI Metropolis, South-East, NIGERIA

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ABSTRACT

Background: In sub-Saharan Africa, caterers and a large population of women are frequently exposed to biomass smoke during cooking. Smoke is associated with reproductive abnormalities in women. Its harmful pollutants including fine particulate matter; carbon monoxide and polycyclic aromatic hydrocarbons may cause defects during gametogenesis, trigger alteration in the release of some reproductive hormones and possibly stimulate immunologic responses leading to vascular injury. **Objective:** This Cross-sectional study examined the effects of exposure to biomass smoke on the reproductive hormones in female caterers in Nnewi using luteinizing hormone (LH), follicle Stimulating hormone (FSH), prolactin (PRL) and estradiol (Es) as markers. **Materials and methods:** Ninety (90) participants (45 female caterers and 45 non-caterers) aged 18 - 40 years old were enrolled for this study. Follicular phase hormonal indices of the subjects were determined using enzyme linked immunosorbent assay. Statistical analysis involved Student's t-test, Pearson's correlation and analysis of variance at $p<0.05$. **Results:** The PRL (ng/ml) level was significantly higher in caterers (19.10±10.11) than non-caterers (15.53±6.35) ($p<0.05$). However, there were non-significant differences between LH, FSH and Es levels in the test subjects compared with the control group ($p>0.05$). Also, non-significant variation existed when the duration of exposure to biomass smoke was compared among the sex hormones amongst caterers for 1-5, 6-10 and >11 years of exposure ($p>0.05$). **Conclusion**: The significantly elevated prolactin level among caterers (although within clinical reference range) suggests the implication of biomass smoke and its constituents in the alteration of female sex hormone.

Keywords: Female sex hormones, Pregnancy, Caterer, Biomass, Aging.
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INTRODUCTION

According to a report from the World Health Organization (WHO) (1), indoor air pollution was the fifth highest cause of death in developing countries with high mortality rates. Many women in Nigeria suffer ailments resulting from the use of firewood which contains harmful pollutants, carcinogenic compounds (benzene, CO, NO_2) and Endocrine disrupting chemicals (EDCs) e.g. particulate matter (PM), dioxin and polycyclic aromatic hydrocarbons (PAH) (2), formed during incomplete combustion of organic materials, including fossil fuels, wood, foods, and tobacco.

Over 98,000 Nigerian women die annually from complications caused by inhalation of firewood smoke (1). The study estimated that if a woman cooks breakfast, lunch and dinner with firewood, it is equivalent to smoking between three (3) and twenty (20) packets of cigarette a day. The death from this sector contributes to 10 percent of global annual death and it is probably higher than that of tuberculosis, HIV/AIDS and malaria combined. A large population of women in sub-Saharan Africa rely on biomass fuel for cooking, which involves the use of solid (coal, fossil fuel; biomass fuel (BMF) like firewood, charcoal, dung and crop residues) and non-solid fuel (kerosene, liquefied petroleum gas (LPG), biogas, ethanol gel, plant oils, dimethyl ether (DME) and electricity (3) to meet the most basic energy needs (cooking, boiling water and heating)(4).

Biomass smoke contains harmful pollutants including fine particulate matter (PM2.5), carbon monoxide, nitrous oxides, sulphur oxides (principally from coal), formaldehyde, PAHs, including carcinogens such as benzo[a]pyrene (5) which pose a risk to the reproductive system. Adverse effects of certain toxicants may not become apparent for years because of the time of their release and the organ of accumulation (6). Women's reproductive physiology, including hormonal function, is reflected by menstrual cycle pattern. Disturbances in any of the stages of menstrual cycle (follicular or luteal phase) may affect the oocyte quality, ovulation, conception, implantation, or survival of the embryo (7). Thus, the menstrual cycle length and the length of follicular and luteal phases are good prognostic factors of reproductive health (8). In addition, Smoke triggers changes in the release of several anterior and posterior pituitary hormones, thereby causing alterations in levels of some sex hormones (9) and possibly simulating an immunologic response leading to vascular injury (10). Some smoke constituents have endocrine disrupting properties (11). Endocrine disruptors can affect every level of the endocrine system (12). They may act by altering hormone synthesis in the endocrine gland or through altering transport of the hormone to the target organ by interfering with the activity of conjugation enzymes or by competing for binding to carrier proteins or antagonizing hormone action. Alternatively, they may act through altering metabolism/excretion of the hormone or through competing with the hormone for binding to a receptor in target cells (13). The transport of hormones is also targeted by certain compounds capable of interacting with the binding sites of hormone, thus competing with endogenous hormones (14).

A large number of households are not aware of the hazards associated with cooking with firewood (15). Little is known about levels of knowledge, attitudes and beliefs of food vendors about the adverse health effects of

biomass smoke exposure in African countries like Nigeria (16).

In Nigeria, especially Southeastern part of the country, data on the effect of firewood smoke on reproductive health status of women is scanty. This study therefore was designed to evaluate the possible effects of biomass smoke inhalationon the reproductive system of female caterers.

MATERIALS AND METHODS

Subjects

A total of 90 Female participants aged 18 – 40 years were randomly recruited from Nnewi metropolis, South-Eastern Nigeria into this cross-sectional study. The test group comprised of 45 adult female commercial caterers constantly exposed to firewood smoke inhalation for at least 2 years prior to this study, while the control group comprised of 45 apparently healthy individual non-caterers who were not exposed to firewood smoke.

Sample Size Determination

The sample size was calculated using the formula described by Taro Yamane (17)

Inclusion and Exclusion criteria

Adult female commercial caterers constantly exposed to firewood smoke for at least 2 years prior to this study, within the age range of 18 – 40 years and apparently healthy adult female non-caterers who were not exposed were included. Those who gave their consent were recruited for the study.

Pregnant adult female commercial caterers, subjects on therapy likely to interfere with the results of the parameters such as non-steroidal anti-inflammatory drugs (NSAID), clomiphene etc, subjects with known chronic conditions of hormones and inflammatory disorders, adult females outside the age range of 18 – 40 years and those who did not give informed consent were excluded from the study.

Ethical approval

The ethical approval for this study was sought for and obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State of Nigeria, with the reference number: NAUTH/CS/66/VOL.10/215/2017/127. The consent of the subjects were sought for and obtained prior to study.

Sample collection

On the follicular (7-13th day) phase of their menstrual cycles, 5 ml of venous blood was obtained aseptically from the ante cubital fossa of each participant using a sterile needle and syringe between 8:00 am and 11:00 am, to control for diurnal variations and dispensed into appropriate plain bottles. The samples were allowed to clot, retract; centrifugation was carried out at 5,000 rpm for 5 minutes and serum separated. Serum samples were labeled and stored in aliquot at -20^{o}C until assay time for the determination of luteinizing hormone (LH), follicle Stimulating hormone (FSH), prolactin (PRL) and estradiol (Es). Information on socio-demography medical history; lifestyle, nature and duration of occupation were obtained using a semi-structured questionnaire.

Biochemical assays Determination of serum Luteinizing hormone

The method for the estimation of Luteinizing Hormone (LH) levels was as described by Jia and Hsueh (18) based on enzyme linked immunosorbent assay (ELISA).

Principle of the Assay

The LH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes mouse monoclonal anti-α-LH for solid phase

(microtiter wells) immobilization, and a mouse monoclonal anti-β-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 minutes incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured photometrically at 450 nm. The concentration of LH is directly proportional to the color intensity of the test sample.

Determination of serum Follicle stimulating hormone

The estimation of Follicle Stimulating Hormone (FSH) levels was based on the ELISA method as described by Jia and Hsueh (18).

Principle of the Assay

The FSH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes mouse monoclonal anti-α-FSH for solid phase (microtiter wells) immobilization, and a mouse monoclonal anti-β-FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 minutes incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of FSH is directly proportional to the color intensity of the test sample.

Assay Procedure for LH and FSH:

After the desired number of coated wells was secured in the holder, 50 µl of standards, specimens, and controls were pipetted into appropriate wells followed by the addition of 100 µl of Enzyme Conjugate Reagent into each well. The wells were gently mixed for 30 seconds followed by its incubation at room temperature (18-25°C) for 45 minutes. The incubation mixture was removed by flicking plate contents into sink. Thereafter, the microtiter wells were rinsed and flicked for 5 times with distilled or de-ionized water. The wells struck sharply onto absorbent paper or paper towels to remove all residual water droplets followed by the addition of 100µl of TMB Reagent into each well. The microtiter wells were gently mixed for 10 seconds, incubated further at room temperature, in the dark, for 20 minutes. The test reaction was put a stop by adding 100µl of Stop Solution to each well, which were gently swirled for 30 seconds until the reaction blue colour changed completely to yellow. Results were read at 450nm with a microtiter plate reader within 15 minutes.

Determination of serum prolactin

The method for the estimation of Prolactin levels was as described by Fariba*et al*(19). The procedure is essentially an enzyme linked immunosorbent assay (ELISA)

ASSAY PRINCIPLE

The Prolactin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a

monoclonal [mouse] antibody directed towards a unique antigenic site on a Prolactin molecule. An aliquot of patient sample containing endogenous Prolactin is incubated in the coated well with enzyme conjugate, which is an anti- Prolactin antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of Prolactin in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Prolactin in the patient sample.

TEST PROCEDURE

Twenty-five (25 µL) of each Calibrator, Control and samples were dispensed into the secured Microtiter wells followed by the addition of 100 µL Enzyme Conjugate into each well which were thoroughly mixed for 10 seconds. Brisk shaking of the contents of the wells was done before rinsing with distilled water (300 µL per well) for 5 times. The wells were struck sharply on absorbent paper to remove residual droplets. 100 µL of Substrate Solution was further added to each well followed by room temperature incubation for 10 minutes. Thereafter, the enzymatic reaction was stopped by the addition of 50 µL of Stop Solution to each well. The absorbance (OD) of each well at 450 nm with a microtiter plate reader was determined.

Determination of serum Luteinizing hormone

The method for the estimation of Luteinizing Hormone (LH) levels was as described by Jia and Hsueh (18) based on enzyme linked immunosorbent assay (ELISA). Prior to the assay, all reagents, references and controls were brought to room temperature after which the micro plate well for each serum reference, control and patient specimen were arranged. Using a pipette, 50µl of the appropriate serum reference, controls and specimen were added to the assigned well followed by 100µl of working reagent (LH-Enzyme Reagent). The micro plate wells were swirled gently for 20 – 30 seconds to mix. The wells were covered and incubated for 60 minutes at room temperature. The contents of the micro plate wells were discarded by decantation. The plate was blotted dry with absorbent paper.

To all wells, 350µl of wash buffer was added followed by decantation (tap and blot). This was repeated two (2) additional times for a total of three (3) washes.

To all wells, 100µl of working substrate solution was added and incubated at room temperature for fifteen (15) minutes followed by 50µl of stop solution to each well and gently mixed for 15 – 20 seconds.

Absorbance was read in each well at 450nm (using a reference wavelength of 620 – 630nm to minimize well imperfections) in a micro plate reader.

Determination of serum Follicle stimulating hormone

The estimation of Follicle Stimulating Hormone (FSH) levels was based on the ELISA method as described by Jia and Hsueh (18). The procedure is the same as described in the determination of LH levels except that in the place of LH-Enzyme Reagent, FSH-Enzyme Reagent was used.

Determination of serum prolactin

The method for the estimation of Prolactin levels was as described byFariba*et al.* (19). Prior to the assay, all reagents, references and controls were brought to room temperature. Using a pipette, 50µl of the appropriate serum reference, controls or specimen was added to the assigned micro plate wells followed by

100µl of working reagent (prolactin-Enzyme Reagent). The micro plate wells were swirled gently for 20 – 30 seconds to mix, covered and incubated for 60 minutes at room temperature.

The contents of the micro plate wells were discarded by decantation. After decanting, the plate was blotted dry with absorbent paper. To the contents of the micro plate wells, 350µl of wash buffer was added followed by decantation. This was repeated two (2) additional times for a total of three (3) washes.

To all wells, 100µl of working substrate solution was added and incubated at room temperature for fifteen (15) minutes followed by addition of 50µl of stop solution. The mixture was mixed gently for 15 – 20 seconds.

Absorbance was read in each well at 450nm (using a reference wavelength of 620 – 630nm to minimize well imperfections) in a micro plate reader.

Determination of serum estradiol

The estimation of estradiol levels was based on the ELISA method as described by Ratcliffe *et al*. (20). Prior to the assay, all reagents, references and controls were brought to room temperature after which the micro plate well for each serum reference, control and patient specimen were arranged accordingly. Using a pipette, 25µl of the appropriate serum reference, controls and specimen was added to the assigned well followed by 50µl of the Estradiol Biotin Reagent. The microplate wells were swirled gently for 20 – 30 seconds to mix and then covered and incubated for 30 minutes at room temperature. To all the contents of micro plate wells, 50µl of Estradiol Enzyme Reagent was added. The micro platewas swirled gently for 20 – 30 seconds to mix, covered and incubated for 90 minutes at room temperature. The contents of the micro plate wells were discarded by decantation. The plate was blotted dry with absorbent paper.

To the contents of the micro plate wells, 350µl of wash buffer was added followed by decantation (tap and blot). This was repeated two (2) additional times for a total of three (3) washes. To all wells, 100µl of working substrate solution was added and incubated at room temperature for twenty (20) minutes followed by 50µl of stop solution. Mixture was mixed gently for 15 – 20 seconds.

Absorbance was read in each well at 450nm (using a reference wavelength of 620 – 630nm to minimize well imperfections) in a micro plate reader.

Statistical analyses

Data analysis was conducted using SPSS version 21.0 (IBM Inc, Chicago, IL). Values were assessed for normality by checking for skewness. Results were expressed as mean ± SD. Student's t-test (Independent t-test) was used to determine significant differences between the means values. Relationship or strength of the association between parameters was assessed using Pearson's correlation coefficient;analysis of variance (ANOVA) was used to compare means across groups. All tests were 2-tailed and p -value of <0.05 was considered significant.

RESULTS

Table 1 shows that the mean serum level of prolactin was significantly higher in caterers (19.10±10.11) when compared with non-caterers (15.53 ± 6.35) ($p=0.045$). On the other hand, the mean levels of LH, FSH and Es were of non-significant difference ($p>0.05$) among the caterers and non-caterers

Table 1: Mean levels of LH, FSH, Prolactin and Estradiol among caterers and non-caterers

Parameters	Caterers (n=45)	Non-caterers (n=45)	Reference Ranges	t-test	p-value
LH (µiU/ml)	4.89 ± 2.06	4.68 ± 2.43	2-8 **(µiU/ml)**	0.436	0.664
FSH (µiU/ml	3.44±1.27	4.92± 2.84	3-10 **((µiU/ml)**	1.127	0.062
PRL (ng/ml)	19.10±10.11	15.53 ± 6.35	3-27 **(ng/ml)**	1.992	0.045*
Es (pg/ml)	32.65 ±16.71	31.60 ± 15.36	< 300 **(pg/ml)**	0.307	0.759

Values are reported as mean ± SD (standard deviation). Mean values are *statistically significant @ $p < 0.05$. Luteinising hormone, (LH), Follicle Stimulating hormone (FSH), Prolactin (PRL), Estradiol (Es), Number of participants (N).

Table 2. **The variation of sex hormone levels based on duration of exposure to biomass inhalation.**

Duration (Years)	LH (µiU/ml)	FSH(µiU/ml	PRL (ng/ml)	Es (pg/ml)
1- 5 (A)	4.78 ± 1.64	3.97±1.33	20.27±10.84	26.46 ±13.29
6 – 10 (B)	5.68 ± 2.35.	3.43± 1.33	19.71 ± 12.31	33.52 ± 19.13
≥ 11 (C)	4.58±2.15	3.00±1.10	18.30±8.69	35.91±17.30
F-value	1.014	2.333	0.148	1.245
P-value	0.372	0.111	0.863	0.299
A vs B	0.888	0.871	1.000	0.925
A vs C	1.000	0.111	1.000	0.384
B vs C	0.518	1.000	1.000	1.000

Values are reported as mean ± SD (standard deviation). Mean values are *statistically significant at $p<0.05$
KEYS: LH = Luteinising hormone, FSH = Follicle Stimulating hormone, PRL = Prolactin, Es = Estradiol, F = ANOVA, N = Number of participants.

Table 3 The duration of exposure among different age groups of the caterers

Age(Yrs)	Duration of Exposure (Years)
18–25 (A)	3.00 ± 1.39
26–33 (B)	4.35 ± 1.60.
34–40 (C)	4.04±2.16
F-value	5.395
P-value	0.006*
A vs B	0.007*
A vs C	0.079
B vs C	1.000

Values are reported as mean ± SD (standard deviation). Mean values are *statistically significant @ $p < 0.05$, Luteinising hormone (LH), Follicle Stimulating hormone (FSH), Prolactin (PRL), Estradiol (Es), N = Number of participants.

Table 2 below shows non-significant difference between the duration of exposure to biomass smoke inhalation amongst caterers and the sex hormones (LH, FSH, PRL and Es) at 1-to-5 years, 6-to-10 years and ≥11 years of exposure to biomass smoke ($p>0.05$).

The mean ages of the caterers and the non-caterers were 30.33 ± 6.85 years and 27.38 ± 4.84 years, respectively.

There was a significant difference between the duration of exposure among subjects of the different age groups (18-25 vs 26-33vs 34-40) years ($p=0.006$); a significant difference also existed between the duration of exposure and the groups aged 18-25 vs 26 – 33 years ($p = 0.007$). However, there were non-significant differences between the duration of exposure and the groups aged (18-25 vs 34-40) years;(26 -33 vs 34-40) years, ($p>0.05$) respectively(Table3).

Figure 1 shows the graph of correlation between the levels of FSH and the duration of exposure in the test group.

The graph revealed a significant inverse correlation ($r = - 0.311$; $p = 0.045$) between the duration of exposure and the FSH among the test group

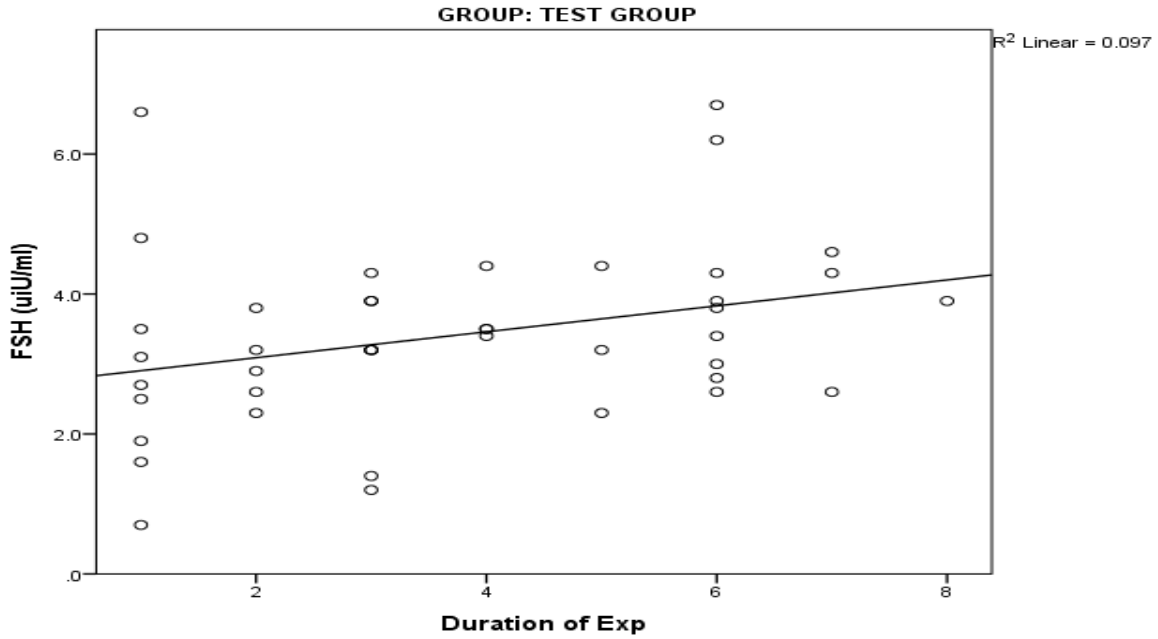

Figure 1: Scatter plot showing the correlation between duration of exposure and FSH level in the study participants (caterers); (r = - 0.311; p = 0.045).

DISCUSSION

Biomass smoke is one of the major air pollutants and contributors of household air pollution worldwide. It is considered one of the leading environmental risk factors of several diseases, including COPD and acute lower respiratory disease, and is thought to cause 4 million deaths annually across the globe (21). The deleterious effects of prolonged exposure to biomass smoke on some hormonal indices have been postulated to have a negative reproductive impact resulting from deranged ovarian function with far reaching effects on sex hormones specifically menstrual cycle, oocyte quality, and risk of miscarriage (21). Hence, this present study was aimed at assessing the impact of biomass smoke on the female sex hormones of caterers who have worked between 1 to 5 years, 6 to 10 years and 11 years above using luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL) and Estradiol (Es) as markers.

There was a significant increase in the serum level of PRL among caterers than non-caterers. This implies that, biomass (a constituent of smoke) can alter PRL concentrations significantly.The higher levels of prolactin (albeit within clinical reference range) in the test group compared to the control may be attributed to the presence of particulate matter (mostly PM10; sulphur and nitrogen oxides, polycyclic aromatic hydrocarbons (PAH), aldehydes, free radicals and non-radical oxidising species) or several of the many constituents of biomass smoke such as carbon monoxide (CO) may modify the episodic secretion of prolactin as evidenced in our research finding. Our study agrees with the findings of Merle *et al.* (22) Xue *et al.*(23) and Seyler *et al.*(24) who stated that exposure to smoke is associated with increased circulating PRL. Smoke stimulates

the release of several anterior and posterior pituitary hormones, acutely increasing the plasma levels of prolactin without significant changes in luteinizing hormone (LH) and follicle-stimulating hormone (FSH); nausea induced by smoke may produce an increase in prolactin.

The non-significant differences recorded between duration of exposure to biomass smoke and female sex hormones agrees with some of the findings of Pasqualotto *et al.*(25) who reported no significant differences in the levels of FSH, LH in smokers. Thismay however be explained in part by genetic factor polymorphism (26), certain individuals are more susceptible to the negative effects of smoke than others. The physical and chemical properties of wood smoke constituents can differ substantially depending on the combustion conditions and fuel used. It is likely that the physicochemical properties of the smoke particles determine their toxicological properties and health effects (27). On the contrary,Twisha*et al.*(28)reported significantly higherlevels of LH, prolactin,lower levels of estrogen (17-beta-estradiol) concentration; significantly lower FSH and LH levels (29)with women using biomass fuelbut differ from the findings of Zumoff*et al.* (30) who reported a significantly higher serum level of estradiol and lower LH in the follicular phase. These differences could be due to the duration of exposure and timing of sample collection which have possible implications for fertility,

CONCLUSION

The significantly elevated mean serum level of prolactin among caterers (although within clinical reference range) suggests the implication of biomass smoke and its constituents in the alteration of female sex hormone. A more clinical trial of biomass exposure on other regularly exposed individuals is suggested.

Conflict of interest

Authors declare no conflict of interest exists.

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