Fountain Journal of Basic Medical and Health Sciences (FUJBMHES), 1(2), 90-101.

Caffeine Exposure during Pregnancy Alters Reproductive Hormone Profiles and Induces Oxidative Stress in F1 Generation Male Piglets

Ladele, M. O.[1], Aderemi, A. V.[2*], & Oyeyipo, I. P.[1]

[1]*Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria.*
[2] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria*

[*]***Corresponding Author**: walerem@yahoo.com; adewale.aderemi@uniosun.edu.ng; +2348065850304*

Abstract

Background: Exposure to environmental toxicants, including caffeine, has been associated with adverse reproductive outcomes, such as reduced fertility. **Objective:** This study investigated the effects of prenatal caffeine exposure on selected antioxidant, hormonal, and lipid profiles in first filial (F1) generation male piglets. **Methodology:** Twenty (20) randomly selected male F1 piglets were used: ten from sows exposed to caffeine during pregnancy (treatment group) and ten from non-exposed sows (control group). Both groups were cohabited with male pigs at a female-to-male ratio of 5:1 and received standard feed and water *ad libitum*. Upon confirmation of pregnancy, 5 mg/kg caffeine was added to the feed of the treatment group and administered until delivery. At three months post-delivery, blood samples were collected from the external jugular vein without sacrificing the animals for analysis of serum lipid profiles, hormone levels, and antioxidant activities. **Results:** The mean weight change was slightly higher in the caffeine-exposed group (20.50 ± 0.4) compared to the control, unexposed group (17.50 ± 0.8), although the observed difference was not statistically significant. The treatment group showed a significant decrease ($p<0.05$) in superoxide dismutase (SOD) activity and an increase in malondialdehyde (MDA) levels ($p<0.05$) compared to the control. Total cholesterol (TC) levels were also higher in the treatment group. While serum testosterone and luteinizing hormone (LH) levels were significantly reduced ($p<0.05$), follicle-stimulating hormone (FSH) levels remained unchanged. **Conclusion/ Recommendations:** Prenatal caffeine exposure induces oxidative stress and disrupts reproductive hormonal profiles in F1 generation male piglets. Further studies are warranted to determine the persistence of these effects in subsequent generations (F2 and F3).

Keywords: Caffeine exposure, male reproductive hormones, antioxidant enzymes, oxidative stress, lipid profile, lipid peroxidation

Introduction

Exposure to environmental and occupational toxicants and progressive changes in many aspects of lifestyle, including dietary habits, have been shown to deteriorate reproductive health, thus affecting the ability of couples to conceive and maintain a healthy pregnancy (Hruska *et al*., 2000). For instance, endocrine-disrupting chemicals (EDCs) such as atrazine, bisphenols, polychlorinated biphenyls, etc., originally designed for agricultural purposes, have been shown to negatively impact reproductive health in women (Piazza & Urbanetz, 2019). Similarly, exposure to heavy metals such as lead has been linked to adverse hormonal disturbances and serious implications

for female reproductive health (Mendola *et al*., 2008). More disturbing is the fact that even at very low doses, these environmental contaminants and EDCs can result in significantly negative biological impacts such as infertility and birth defects (Fucic *et al*., 2021), although their overall mechanisms of action are still subjects of research (Ghosh *et al*., 2022).

Caffeine, a widely consumed xenobiotic during pregnancy, has been reported to have developmental effects at moderate doses (Narod *et al*., 1991), and its intake at moderate doses of between 150 and 300 mg/day is a risk factor in infertility, fetal growth impairment, and fetal loss (Dlugosz & Bracken, 1992). Its continuous consumption throughout gestation exposes the fetus to caffeine and its metabolite, which may alter development, growth, and physiological processes in adulthood (Bakker *et al*., 2010). Research has further shown that caffeine has some biological effects, including: central nervous system stimulation, increased secretion of catecholamine, relaxation of smooth muscles, and stimulation of heart rate, with moderate intake playing a protective role against some cardiovascular diseases and on metabolism of carbohydrates and lipids (Cano-Marquina *et al*., 2013). With about three-quarters of women consuming it during pregnancy (Sengpiel *et al*., 2013)It is one of the most widely consumed xenobiotics in pregnancy, with the potential to adversely affect the developing fetus (Lakin *et al*., 2023).

Caffeine (1, 3, 7- trimethylxanthine) is a plant alkaloid with a chemical structure of $C_8H_{10}N_4O_2$ and a molecular weight of 194.19. Caffeine is a bitter white powder, which in its pure form is structurally identical to purines. It is rapidly absorbed in humans, with 99% being absorbed within 45 minutes of ingestion (Liguori *et al*., 1997). Caffeine is found in several prescription drugs such as stimulants, headache treatments, diet pills, cold and flu remedies, and naturally occurring alkaloids present in seeds, leaves and fruits of various plants such as coffee, cocoa beans, kola nuts and tea leaves. Caffeine is also present in a wide variety of foods and beverages, including energy drinks, with the vast majority of its consumers unaware of its associated health hazards(Lone *et al*., 2023). Caffeine may further act indirectly by affecting the hypothalamo-pituitary-gonadal system and by exhibiting its toxic effect on the germinative epithelium that alters Sertoli cells' glycolytic and oxidative profile, thereby affecting male reproductive potential at adulthood (Dias *et al*., 2015).

Oxidative stress is defined as an imbalance between reactive oxygen species and the antioxidant defence mechanism of a cell, resulting in excessive production of oxygen metabolites (Sudha *et al*., 2001). Pregnancy is associated with alterations in physiologic and metabolic functions that is accompanied by high metabolic demand and increased requirements for tissue oxygen (Soma-Pillay *et al*., 2016). Reactive oxygen species (ROS) produced by exogenous and endogenous factors from oxidative stress are highly reactive oxygen derivatives with half-lives in the nano to milliseconds range, which have been implicated in altering male reproductive functions (Agarwal & Prabakaran, 2005). However, among the primary exogenous factors that cause reactive oxygen species (ROS) production are alcohol consumption, lifestyle modifications, cigarette smoking, technological advancements, and stress (Sullivan & Chandel, 2014).

Epidemiological studies on caffeine and its effects on reproductive outcomes have been somewhat conflicting (Grosso & Bracken, 2005; Dorostghoal *et al*, 2012). The objective of the study was to investigate whether exposure to caffeine in pregnancy has negative reproductive consequences on the first filial (F1) generation male offspring or not, using pregnant sows as models to investigate the effects of prenatal caffeine exposure on the reproductive hormones,

oxidative stress, and lipid profiles in male F1 generation piglets.

Materials and Methods

Animal grouping and experimental design

In the 1st phase of this study, a total of twenty (20) female and 4 male pigs were randomly grouped into control and treated groups, with 10 female and 2 male sows in each group, and mated giving a female to male ratio of 5:1. Both groups received water *ad libitum* and standard animal feeds procured from Wemikun Farms and Agro- allied Industry, Owode-Ede, Nigeria, until pregnancy was established. Thereafter, the treatment group received 5 mg/kg of caffeine mixed with 2.5kg/day until farrowing for a period of 114 days throughout gestation, before it was gradually increased to 7kg/day by day 7 of lactation in two divided doses. The control continued to receive the standard animal feed and water only. Animals were housed in pens with slatted flooring in temperature-controlled rooms maintained at a normal temperature of 25ºC. The boar was cohabited with sows once estrus was confirmed by the detection of edematous vulva, vaginal discharge, and frequent urination, and lasted for a duration of about 2-3 days. Changes in behaviour were also observed in the sows after mating, with sows not allowing the boar to mount. In the 2nd phase of the study, ten (10) male F1 generation offspring were randomly selected from both the control and the treated groups and tagged at birth for easy identification. The animals were weighed at birth and monitored over a period of 3 months before they were weighed again, and samples were collected for biochemical assays. The study was carried out in accordance with the University's ethics guidance on the use of animals for laboratory studies.

Specimen collection

Blood was collected at 3 months post-delivery from the F1 generation male piglets through jugular vein puncture for biochemical estimations. Blood collection was spun at 3,000 rpm for 15 min and the serum obtained was used for the determination of hormone assay and oxidative stress parameters, and lipid profile

Hormone and oxidative stress assay

The serum testosterone, LH, and FSH concentration estimations were carried out using the tube-based Enzyme Immunoassay (E.I.A) method. The E.I.A. testosterone test kit produced by Immunometric (U.K.) Ltd was obtained from Nzemat (Nigeria) Limited, Akoka, Yaba, Lagos. The tube-based Enzyme immunoassay showed a high sensitivity, specificity and accuracy for the detection of testosterone, FSH and LH with intra-assay and inter-assay coefficient of variation for the respective hormone in the range values acceptable for E.I.A.

Oxidative stress was assessed as follows: Glutathione peroxidase (GP_X) was measured as previously described, with slight modification (Sun & Zigman, 1978). Superoxide dismutase (SOD) was measured according to Sun and Zigman (Sun & Zigman, 1978). The serum Catalase (CAT) activity was measured as previously done by Goth (Goth, 1991), while Malondialdehyde (MDA) level was measured according to Uchiyama and Mihara (Uchiyama & Mihara, 1978).

Serum lipid profile biomarkers

Total cholesterol (TC), triglyceride, high-density lipoprotein cholesterol (HDL-cholesterol) and low-density lipoprotein-cholesterol (LDL-cholesterol) in the serum obtained were measured by the enzymatic colourimetric method as previously described (Hadi *et al*., 2023). This principle was based on colour formation after enzymatic hydrolysis and oxidation. Quinoneimine which was an indicator was formed from hydrogen peroxide and 4-amino- antipyrine in the presence of phenol. The VLDL-cholesterol was estimated from the TC, TG and HDL-cholesterol using the Friedewald equation:

$$\text{VLDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/5) - (1)$$

Statistical Analysis

Data were expressed as Mean ± Standard error of mean (SEM), and analysis was done using an unpaired *t*-test and a Mann-Whitney U-test with Graphpad Prism version 10.0. Statistical significance was considered at $p<0.05$. While the t-test assumes equal variances between the two groups being compared, normality was assessed using built-in tests in GraphPad Prism (e.g., Shapiro-Wilk test). Where data failed to meet normality assumptions, the non-parametric Mann-Whitney U-test was applied as an alternative.

Results

Effect of maternal exposure to caffeine on weight

Table 1 shows the comparison in the net weight gain between the F1 male generation piglets prenatally exposed to caffeine and the control. The mean weight change was slightly higher in the caffeine-exposed group (20.54 ± 0.40) compared to the control, unexposed group (17.51 ± 0.78), although the observed difference was not statistically significant.

Effects on lipid profiles

The result showed a statistically significantly higher serum total cholesterol values in the offspring of the treated sows, while serum triglyceride level was unaffected. On the other hand, HDL-cholesterol and LDL-cholesterol serum levels were insignificantly raised in the offspring of the treated sows (Table 2).

Effects on antioxidant enzyme activities

Intrauterine caffeine exposure showed a statistically insignificant decrease ($p>0.05$) in the antioxidant activities of GPx and CAT in the treated group compared to the control (Figures 1A & C). However, a statistically significant decrease ($p<0.05$) in serum antioxidant activity level of SOD and a significant increase ($p<0.05$) in MDA concentration were observed in the treated group compared to the control (Figures 1B and D).

Effects on reproductive hormone profiles

The study further assessed the effects of prenatal caffeine exposure on the male reproductive hormone profiles of the F1 generation male piglets, and the results are presented in Figure 2. A significant decrease ($p<0.05$) in serum level of both testosterone and LH was observed, while that of FSH was insignificantly affected.

Table 1: Body weight measurements of male offspring of sows exposed to caffeine. Units are in Kg, while n represents the sample size for the study.

Group/ Parameters	Control (n=10)	Treated (n=10)
Initial Weight (g)	2.12 ± 0.46	2.36 ± 0.74
Final Weight (g)	19.63 ± 1.24	22.93 ± 1.14
Change in Weight (g)	17.51 ± 0.78	20.54 ± 0.40

Table 2: Serum Lipid profile of male offspring of pregnant sows exposed to Caffeine

	Control (n = 10)	Treated (n = 10)
Total Cholesterol (mmol/l)	2.02 ± 0.07	2.76 ± 0.27**
Triglyceride (mmol/l)	1.80 ± 0.08	1.78 ± 0.09
LDL-cholesterol (mmol/l)	1.20 ± 0.10	1.46 ± 0.08
HDL-cholesterol (mmol/l)	0.60 ± 0.04	0.88 ± 0.24
VLDL-cholesterol (mmol/l)	1.06 ± 0.06	1.52 ± 0.20**

** showed statistically significant values ($p<0.05$) from the control. Values are measured in mmol/L. n – represents the sample size for the study.

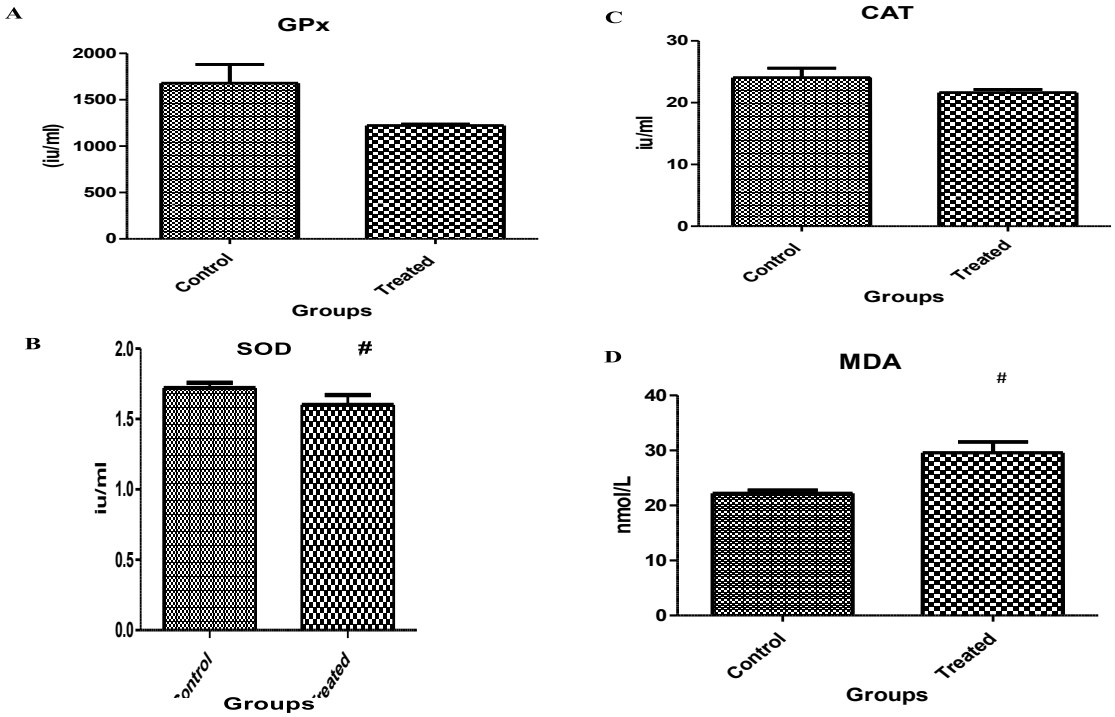

Figure 1: Antioxidant enzyme activities in F1 generation male sows prenatally exposed to caffeine. A-D) shows the serum antioxidant activity level of GPx, SOD, and CAT, and MDA concentration in the F1 generation male piglets, respectively. # Indicates values that were statistically significant compared to the control. Results indicate that only the SOD and MDA values in the treated groups, denoted with (#) are statistically significantly different ($p<0.05$) from the control. Units are in iu/ml for SOD, CAT and GPx, while MDA concentration is reported as nmol/L. SOD, CAT, GPx and MDA stand for superoxide dismutase, catalase, glutathione peroxidase and Malondialdehyde, respectively.

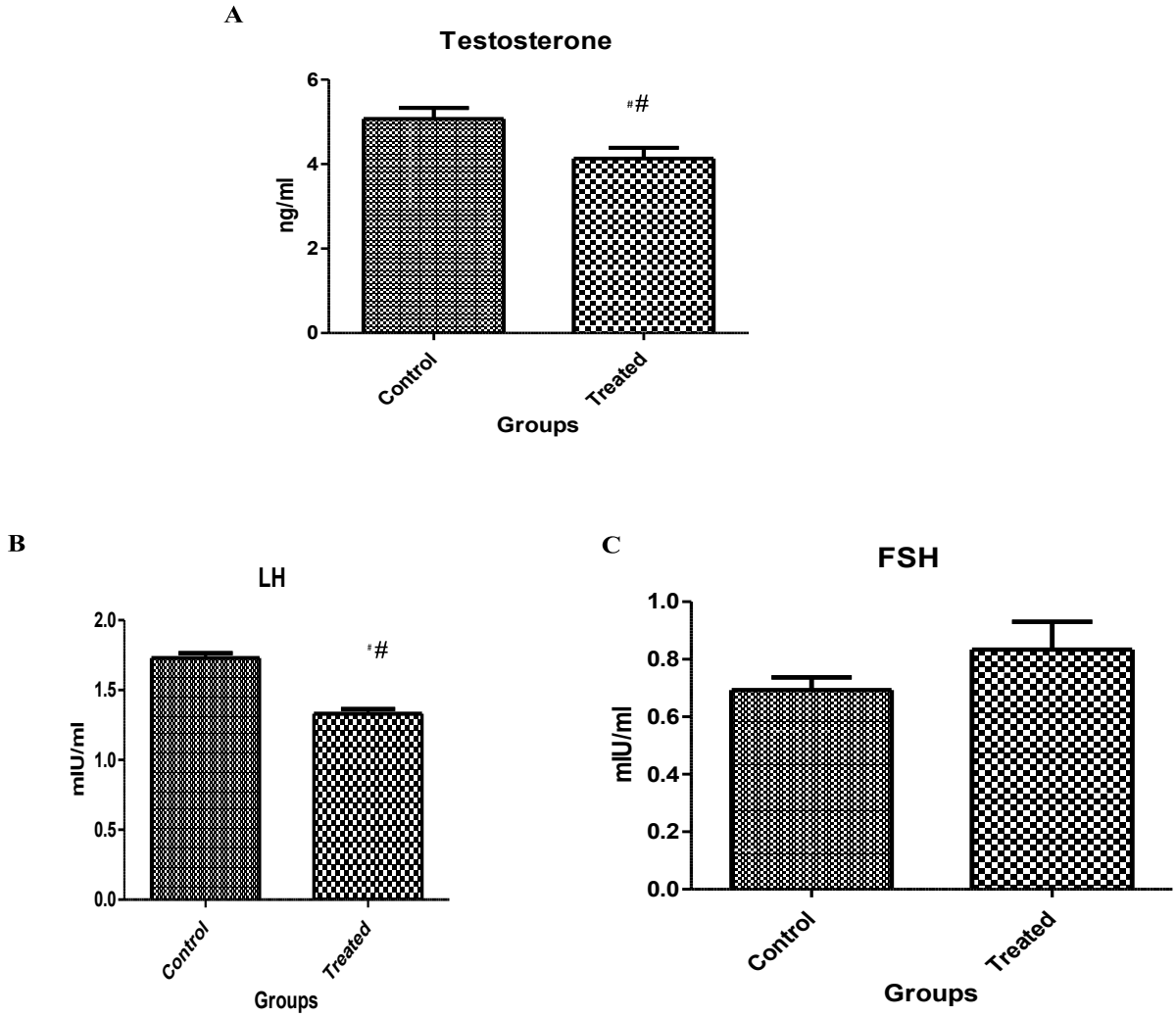

Figure 2: Effects of maternal exposure to caffeine on male reproductive hormones of F1 generation piglets. A-C represent the serum testosterone, LH, and FSH levels, respectively. Results indicate that only the testosterone and LH values in the treated groups, denoted with (#), are statistically significantly different ($p<0.05$) from the control. Units are in ng/ml for testosterone, while both LH and FSH are reported as mIU/ml. LH and FSH stand for luteinizing hormone and follicle-stimulating hormone, respectively.

Discussion

The potential effects of caffeine on health have been well documented due to its wide consumption by people of reproductive age. Beverages such as energy drinks, tea and coffee have a higher concentration of caffeine (Reissig *et al*., 2009) and the average daily consumption is estimated to be 5 mg/kg per individual, reaching a plasma concentration of 50 mM (Blanchard & Sawers, 1983; Chou & Benowitz, 1994). Studies have shown interspecies differences in caffeine metabolic pathways (Blanchard & Sawers, 1983), which could influence the elimination pharmacokinetic parameters, leading to a much higher elimination half-life of caffeine in sows (13.3 h) than in humans (4.2 h) (Chou & Benowitz, 1994).

The present study sought to establish if exposure of sows to caffeine affects reproductive

hormones, oxidative biomarkers and lipid profile of their male offspring during development. The results at a dose of 5 mg/kg body weight of maternal caffeine exposure throughout gestation showed a significant reduction in reproductive hormones and a significant increase in oxidative biomarkers in the offspring of the treated sows. Reports of transient low birth weight of the fetus due to continuous intrauterine caffeine exposure have been well documented (Bracken *et al*., 2003; Martin & Bracken, 1987). This is due to increased maternal absorption of calcium and iron from food intake during pregnancy, depriving the fetus of the needed nutrients for growth and development. However, in this present study, we recorded a comparative increase in birth weight of the piglets in the treated group compared to the control group. The weight gain observed could be attributed to disruption in the gut microbiomes in the piglets as a result of caffeine alteration, leading to changes in the way the body processes and stores energy, contributing to weight gain (Zhang *et al*., 2020). The gut microbiome is known to be the second human genome that may affect the body's energy balance (Turnbaugh *et al*., 2006). Its imbalance can alter gut barriers and gut-associated lymphoid tissues, which allows bacterial components to pass through the intestinal wall. Dubosiella is a bacterium present in the gut microbiomes that is significantly elevated in response to caffeine in humans and animal models. Although still not well studied, Dubosiella has been negatively linked to most inflammatory factors and obesity, resulting in weight gain (Obanda *et al*., 2021).

Glutathione peroxidase (GPx) is an enzyme that reduces lipid hydroperoxides to the respective alcohol and hydrogen peroxide to water. Our study showed a decrease in glutathione peroxidase (GPx) in the offspring of the treated group compared to the control group. This could be due to epigenetic modification from continuous caffeine exposure that could affect the glutathione peroxidase (GPx) gene expression in the offspring of the treated group and early life exposure to lifestyle modification, including diet-induced long-term changes in DNA methylation that can impact individual health and age-related diseases during development and throughout life (Lillycrop *et al*., 2014).

The SOD is a chain-breaking antioxidant, while CAT functions as a preventive antioxidant with both enzymes performing a protective role against the deleterious effects of lipid peroxidation (Dinkova-Kostova, 2005). Catalase (CAT) scavenges hydrogen peroxide (H_2O_2) that is generated by free radicals or by SOD conversion of superoxide anions to water (Ribière *et al*., 1992). The significant decrease in SOD is attributed to the reduction of blood flow to the placenta. This may lead to insufficient oxygen and nutrient supply to the fetus as well as altered metabolic and antioxidant processes that reduce superoxide dismutase activities in rat offspring (Chen *et al*., 2020). CAT, on the other hand, showed a reduction in activity due to overutilization of cellular antioxidants that clear up free radicals produced during lipid peroxidation (Frangogiannis, 2006).

The terminal product of lipid peroxidation is MDA, which serves as its index. It is produced through the process of prostaglandin synthesis (Marnett, 2002) and by the peroxidation of the membrane polyunsaturated fatty acid (Esterbauer, 1996). This increased significantly in the offspring of the treated group compared to the control group. The increase is an indication of oxidative stress in the treated group, and an imbalance between the antioxidant defence mechanism in the body and the generation of Reactive Oxygen Species (ROS), which is detrimental to proteins and lipids, resulting in necrosis in living cells (Halliwell, 1994).

A significant increase in total cholesterol (TC) and VLDL-cholesterol observed in the offspring of the treated group is an indication of hyperlipidemia (Chowdhury *et al*., 2016). Hyperlipidemia is an increase in the blood lipid

that could lead to cardiac disease resulting from atherosclerosis in the vessels surrounding the heart. The LDL-cholesterol levels, which represent the so-called 'bad cholesterol', and the HDL-cholesterol levels, representing the 'good cholesterol,' are not significantly different in both the control and the treated groups **(**Table 2**).**

The reproductive hormones (testosterone, LH, and FSH) are controlled by complex interactions within the hypothalamo-pituitary-testicular system, and caffeine may exhibit its toxic effect on the germinative epithelium that alters Sertoli cells' glycolytic and oxidative profile, thereby affecting male reproductive potential at adulthood (Dias *et al*., 2015). Testosterone is required for the masculinization and development of the male reproductive organs. Its production is controlled by the influence of LH on the Leydig cells, which produce testosterone in the testes. The hypothalamo-pituitary system controls the function of LH on the Leydig cells. The significant decrease in the serum level of both testosterone and LH in the present study may be due to the toxic effect of caffeine on the hypothalamo-pituitary axis that negatively impacts the functional capacity of the cells of the testes to secrete the male reproductive hormone. The extent of the toxic effect of testicular damage is directly related to the duration of drug consumption, as reported by Vigezzi *et al*., (2006), which was throughout gestation. Rhind *et al*. (2001) also showed that exposure to environmental insults during the period of organogenesis may cause defects that can permanently reduce the functional capacity of the testicular organ, thus corroborating our findings in this study. The FSH level was, however, insignificantly altered in the treated group, compared with the control.

While the toxicological mechanisms employed remain largely unknown, the observed antioxidant effects of caffeine in this study may have been exerted via interference with gene expression and epigenetic regulations, antagonistic effects on the adenosine receptors or some complex intracellular transduction signalling pathways (Ősz *et al*., 2022). On the other hand, its hormonal imbalance effects may have arisen from its direct damaging effects on the testicular cellular architectures or indirectly via its disruption of the hypothalamo-pituitary-testicular (HPT) axis (Joshua *et al*., 2017).

Conclusion

Taken together, this study showed that maternal caffeine exposure could be detrimental to the offspring through alterations in the reproductive hormones and oxidative biomarkers. Further studies are required on these models at adulthood as a continuation to examine if these alterations are transient or permanent, possibly through F2 and F3 generations of offspring.

Recommendations

This study shows that caffeine exposure during pregnancy can have a significant negative impact on the health of the newborn, including those relating to their ability to achieve conception in adult life. There is therefore a need for sensitization and awareness creation among women to avoid undue exposure to this toxicant, especially during pregnancy. Future studies that are aimed at understanding how the current effects in the F1 generation male piglets' impact on the reproductive health, especially in relation to fertility outcomes in the future, are recommended.

Limitations of the study

The authors recognize that, whereas the results show the deleterious effects of maternal caffeine exposure on the F1 generation piglets, these cannot be directly extrapolated to human beings. Additionally, histological studies of the affected reproductive organs needed to further confirm the toxicological effects of caffeine exposure are unavailable in the current study because the animals were not sacrificed. Future studies will have to consider this. Another limitation of the study is that we did not specifically measure parameters such as weight gain, stress markers,

hormone changes, etc. in the mother, which may indirectly affect the fetus, but we are aware that caffeine exposure can prolong gestation and increase maternal weight.

Funding
The authors received no funding from any individuals or corporate organizations for this study. It was fully funded by the authors

Informed consent
Not required.

Acknowledgements
We thank Dr. T.G. Atere of the Medical Biochemistry Department, Osun State University, for his technical support during the study and report writing

Conflict of interest
Authors declare no conflict of interest

References

Agarwal, A., & Prabakaran, S. A. (2005). Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology*, 43(11), 963–974.

Bakker, R., Steegers, E. A. P., Obradov, A., Raat, H., Hofman, A., & Jaddoe, V. W. V. (2010). Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: The Generation R Study. *American Journal of Clinical Nutrition*, 91(6), 1691–1698. https://doi.org/10.3945/ajcn.2009.28792

Blanchard, J., & Sawers, S. J. A. (1983). The absolute bioavailability of caffeine in man. *European Journal of Clinical Pharmacology*, *24*(1), 93–98. https://doi.org/10.1007/BF00613933

Bracken, M. B., Triche, E. W., Belanger, K., Hellenbrand, K., & Leaderer, B. P. (2003). Association of maternal caffeine consumption with decrements in fetal growth. *American Journal of Epidemiology*, 157(5), 456–466. https://doi.org/10.1093/aje/kwf220

Cano-Marquina, A., Tarín, J. J., & Cano, A. (2013). The impact of coffee on health. *Maturitas*, 75(1), 7–21. https://doi.org/10.1016/j.maturitas.2013.02.002

Chen, F., Wang, H., Chen, J., Liu, Y., Wen, W., Li, Y., & Huang, X. (2020). Lactobacillus delbrueckii Ameliorates Intestinal Integrity and Antioxidant Ability in Weaned Piglets after a Lipopolysaccharide Challenge. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/10.1155/2020/6028606

Chou, T. M., & Benowitz, N. L. (1994). Caffeine and coffee: effects on health and cardiovascular disease. *Comparative Biochemistry and Physiology. Part C: Comparative*, 109(2), 173–189. https://doi.org/10.1016/0742-8413(94)00048-F

Chowdhury, S. S., Lecomte, V., Erlich, J. H., Maloney, C. A., & Morris, M. J. (2016). Paternal high fat diet in rats leads to renal accumulation of lipid and tubular changes in adult offspring. *Nutrients*, 8(9). https://doi.org/10.3390/nu8090521

Dias, T. R., Alves, M. G., Bernardino, R. L., Martins, A. D., Moreira, A. C., Silva, J., Barros, A., Sousa, M., Silva, B. M., & Oliveira, P. F. (2015). Dose-dependent effects of caffeine in human Sertoli cells metabolism and oxidative profile: Relevance for male fertility. *Toxicology*, *328*, 12–20. https://doi.org/10.1016/j.tox.2014.12.003

Dinkova-Kostova, A. (2005). Protection Against Cancer by Plant Phenylpropenoids: Induction of Mammalian Anticarcinogenic Enzymes. *Mini-Reviews in Medicinal Chemistry*, 2(6), 595–610. https://doi.org/10.2174/1389557023405558

Dlugosz, L., & Bracken, M. B. (1992). Reproductive effects of caffeine: A review and theoretical analysis. *Epidemiologic Reviews*, 14(1), 83–100. https://doi.org/10.1093/oxfordjournals.epirev.a036093

Dorostghoal, M., Majd, N. E., & Nooraei, P. (2012). Maternal caffeine consumption has irreversible effects on reproductive parameters and fertility in male offspring rats. *Clinical and Experimental Reproductive Medicine*, 39(4), 144–152. https://doi.org/10.5653/cerm.2012.39.4.144

Esterbauer, H. (1996). Estimation of peroxidative damage. A critical review. *Pathologie Biologie*, 44(1), 25–28.

Frangogiannis, N. (2006). Comprehensive Invited Review. *Antioxidants & Redox Signaling*, 8(11 & 12), 1907–1939.

Fucic, A., Duca, R., Galea, K. S., Maric, T., Garcia, K., Bloom, M., Andersen, H. R., & Vena, J. (2021). Reproductive health risks associated with occupational and environmental exposure to pesticides. *International Journal of Environmental Research and Public Health*, 18(12). https://doi.org/10.3390/ijerph18126576

Ghosh, A., Tripathy, A., & Ghosh, D. (2022). Impact of Endocrine Disrupting Chemicals (EDCs) on Reproductive Health of Human. *Proceedings of the Zoological Society*, 75(1), 16–30. https://doi.org/10.1007/s12595-021-00412-3

Góth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196(2–3), 143–151. https://doi.org/10.1016/0009-8981(91)90067-M

Grosso, L. M., & Bracken, M. B. (2005). Caffeine metabolism, genetics, and perinatal outcomes: A review of exposure assessment considerations during pregnancy. *Annals of Epidemiology*, 15(6), 460–466. https://doi.org/10.1016/j.annepidem.2004.12.011

Günzler, W. A., Kremers, H., & Floha, L. (1974). An improved coupled test procedure for glutathione peroxidase (EC 1-11-1-9-) in blood. *Z Klin Chem Klin Biochem.*, *12*, 444–448.

Hadi, W., T. Khudair, T., & G. Al-Fartosi, K. (2023). Level of lipid profile and liver enzyme of diabetic male rats induced by streptozotocin treated with forxiga. *3C Empresa. Investigación y Pensamiento Crítico*, 12(01), 273–288. https://doi.org/10.17993/3cemp.2023.120151.273-288

Halliwell, B. (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or Consequence? *The Lancet*, *344*, 721–724.

Hruska, K. S., Furth, P. A., Seifer, D. B., Sharara, F. I., & Flaws, J. A. (2000). Environmental factors in infertility. *Clinical Obstetrics and Gynecology*, 43(4), 821–829. https://doi.org/10.1097/00003081-200012000-00014

Joshua, O., Sunday, O., John, O. A., Titilope, O., & Owolani, J. O. (2017). Excessive Caffeine Intake Disrupts Testicular Architecture, Spermatogenesis and Hormonal Levels in Experimental Wistar Rats. *IOSR Journal of Environmental Science*, 11(10), 8–18. https://doi.org/10.9790/2402-1110030818

Lakin, H., Sheehan, P., & Soti, V. (2023). Maternal Caffeine Consumption and Its Impact on the Fetus: A Review. *Cureus*, 15(11).

https://doi.org/10.7759/cureus.48266

Liguori, A., Hughes, J. R., & Grass, J. A. (1997). Absorption and subjective effects of caffeine from coffee, cola and capsules. *Pharmacology Biochemistry and Behavior*, 58(3), 721–726. https://doi.org/10.1016/S0091-3057(97)00003-8

Lillycrop, K. A., Hoile, S. P., Grenfell, L., & Burdge, G. C. (2014). DNA methylation, ageing and the influence of early life nutrition. *Proceedings of the Nutrition Society*, 73(3), 413–421. https://doi.org/10.1017/S0029665114000081

Lone, A., Alnawah, A. K., Hadadi, A. S., Alturkie, F. M., Aldreweesh, Y. A., & Alhedhod, A. T. (2023). Coffee Consumption Behavior in Young Adults: Exploring Motivations, Frequencies, and Reporting Adverse Effects and Withdrawal Symptoms. *Psychology Research and Behavior Management*, 16(September), 3925–3937. https://doi.org/10.2147/PRBM.S427867

Marnett, L. J. (2002). Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, *181–182*, 219–222. https://doi.org/10.1016/S0300-483X(02)00448-1

Martin, T. R., & Bracken, M. B. (1987). The association between low birth weight and caffeine consumption during pregnancy. *American Journal of Epidemiology*, 126(5), 813–821. https://doi.org/10.1093/oxfordjournals.aje.a114718

Mendola, P., Messer, L. C., & Rappazzo, K. (2008). Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult female. *Fertility and Sterility*, 89(2 SUPPL.), 81–94. https://doi.org/10.1016/j.fertnstert.2007.12.036

Narod, S. A., de Sanjosé, S., & Victora, C. (1991). Coffee during pregnancy: A reproductive hazard? *American Journal of Obstetrics and Gynecology*, 164(4), 1109–1114. https://doi.org/10.1016/0002-9378(91)90597-K

Obanda, D. N., Keenan, M. J., Page, R., Raggio, A. M., Taylor, C. M., Marx, B. D., Stout, R. W., Guice, J., Luo, M., Welsh, D. A., Coulon, D., & Husseneder, C. (2021). Gut Microbiota Composition and Predicted Microbial Metabolic Pathways of Obesity Prone and Obesity Resistant Outbred Sprague-Dawley CD Rats May Account for Differences in Their Phenotype. *Frontiers in Nutrition*, 8(December), 1–17. https://doi.org/10.3389/fnut.2021.746515

Ősz, B. E., Jîtcă, G., Ștefănescu, R. E., Pușcaș, A., Tero-Vescan, A., & Vari, C. E. (2022). Caffeine and Its Antioxidant Properties—It Is All about Dose and Source. *International Journal of Molecular Sciences*, 23(21). https://doi.org/10.3390/ijms232113074

Piazza, M. J., & Urbanetz, A. A. (2019). Environmental toxins and the impact of other endocrine-disrupting chemicals in women's reproductive health. *Jornal Brasileiro de Reproducao Assistida*, 23(2), 154–164. https://doi.org/10.5935/1518-0557.20190016

Reissig, C. J., Strain, E. C., & Griffiths, R. R. (2009). Caffeinated energy drinks-A growing problem. *Drug and Alcohol Dependence*, 99(1–3), 1–10. https://doi.org/10.1016/j.drugalcdep.2008.08.001

Rhind, S. M., Rae, M. T., & Nigel Brooks, A. (2001). Effects of nutrition and environmental factors on the fetal

programming of the reproductive axis. *Reproduction*, 122(2), 205–214. https://doi.org/10.1530/rep.0.1220205

Ribière, C., Hininger, I., Rouach, H., & Nordmann, R. (1992). Effects of chronic ethanol administration on free radical defence in rat myocardium. *Biochemical Pharmacology*, 44(8), 1495–1500. https://doi.org/10.1016/0006-2952(92)90463-S

Sengpiel, V., Elind, E., Bacelis, J., Nilsson, S., Grove, J., Myhre, R., Haugen, M., Meltzer, H. M., Alexander, J., Jacobsson, B., & Brantsæter, A. L. (2013). Maternal caffeine intake during pregnancy is associated with birth weight but not with gestational length: Results from a large prospective observational cohort study. *BMC Medicine*, *11*(1), 42. https://doi.org/10.1186/1741-7015-11-42

Soma-Pillay, P., Nelson-Piercy, C., Tolppanen, H., & Mebazaa, A. (2016). Physiological changes in pregnancy. *Cardiovascular Journal of Africa*, 27(2), 89–94. https://doi.org/10.5830/CVJA-2016-021

Sudha, K., Rao, A. V., & Rao, A. (2001). Oxidative stress and antioxidants in epilepsy. *Clinica Chimica Acta*, 303(1–2), 19–24. https://doi.org/10.1016/S0009-8981(00)00337-5

Sullivan, L. B., & Chandel, N. S. (2014). Mitochondrial reactive species and cancer. *Cancer & Metabolism*, 2(17), 1–12.

Sun, M., & Zigman, S. (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry*, 90(1), 81–89. https://doi.org/10.1016/0003-2697(78)90010-6

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122), 1027–1031. https://doi.org/10.1038/nature05414

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, 86(1), 271–278. https://doi.org/10.1016/0003-2697(78)90342-1

Vigezzi, P., Guglielmino, L., Marzorati, P., Silenzio, R., De Chiara, M., Corrado, F., Cocchi, L., & Cozzolino, E. (2006). Multimodal drug addiction treatment: A field comparison of methadone and buprenorphine among heroin- and cocaine-dependent patients. *Journal of Substance Abuse Treatment*, 31(1), 3–7. https://doi.org/10.1016/j.jsat.2006.03.007

Zhang, H., Chen, Y., Li, Y., Jia, P., Ji, S., Chen, Y., & Wang, T. (2020). Protective effects of pterostilbene against hepatic damage, redox imbalance, mitochondrial dysfunction, and endoplasmic reticulum stress in weanling piglets. *Journal of Animal Science*, 98(10), 1–16. https://doi.org/10.1093/JAS/SKAA328

Citation:

Ladele, M.O., Aderemi, A.V., & Oyeyipo, I.P. (2025). Caffeine Exposure during Pregnancy Alters Reproductive Hormone Profiles and Induces Oxidative Stress in F1 Generation Male Piglets. *Fountain Journal of Basic Medical and Health Sciences (FUJBMHES), 1(2),* 90-101.