ORIGINAL RESEARCH

Assessment of the Pituitary-Ovarian Axis Function in Response to Caffeine Ingestion in Pubertal *Sprague-Dawley* Rats

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ABSTRACT

Background: Some complications of infertility arise from various factors such as environmental pollution, occupational toxicants, including dietary factors. Caffeine consumption in our diet, from coffee, tea and cigarette of all kinds, has impacted reproductive health.
Method: Eighteen mature female *Sprague-Dawley* rats, with weights ranging between 140-184 g, were used. The rats were divided into three groups (in no order; six rats per group): Control group, 5mg caffeine administered rats and 10 mg caffeine administered rats. The rats were smeared before administration of caffeine to ensure they are normal cycling rats. Caffeine, with concentrations of 5 ml/kg and 10 ml/kg, was administered to the appropriate groups for four weeks. The rats were smeared, after four weeks of administration, to check their phase in the oestrous cycle. Samples of their ovaries were weighed and corpus luteum counted. Serum level of follicular stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) obtained from their blood samples were collected and determined. TSH level was significantly reduced at dioestrous phase in 5 mg group.
Results: Both serum FSH and LH were significantly reduced at dioestrus in both 5 mg and 10 mg groups. Caffeine expressed dose dependent stimulatory effect on TSH while it precipitated a reduction in serum FSH and LH level at all doses used during dioestrus. The 10 mg stimulated FSH and LH secretion at oestrous phase which enhances growth and maturation of follicles.
Conclusion: Caffeine thus modulates the activities of the pituitary gland in regulating female reproduction

Keywords: Pituitary-ovarian axis, Caffeine, Gonadotropin, Corpus luteum, Sleep deprivation, *Sprague-Dawley* rats

INTRODUCTION

Environmental pollution and its attendant health challenges on man and his livestock have been a major public health concern and has generated enormous research over the years[1]. Most environmental pollutants that double as endocrine disruptors usually disrupt the functional ability and capacity of the hypothalamo-pituitary regulatory mechanism responsible for neuroendocrine control of the body system. The pituitary is carefully concealed and protected in the brain[2]and it serves as the master endocrine gland in the body. The pituitary secretes several hormones among which are the gonadotropes (FSH and LH) with direct effect on the ovaries. In the female

reproductive system, FSH and LH act primarily to stimulate the ovaries to produce oestrogen and inhibin, which are involved in the regulation of the ovarian cycle, menstrual cycle (or oestrous cycle for lower animals).

In recent past, concerns have been raised in respect of the increasing reproductive disorders among men and women of reproductive age[3]. Some of these disorders have been associated with changes in lifestyle including dietary habits and exposures to environmental and occupational toxicants[4]. Among such dietary factors of interest is the consumption of caffeine. There are reports of increased consumption of high levels of dietary caffeine in children and adolescents[5, 6]. Commonly consumed food such as coffee, tea, carbonated drinks, etc. are common sources of caffeine. The total daily intake of caffeine from all sources is estimated to be about 3-7 mg/kg per day or 200 mg/day[7]. Caffeine consumption has been associated with early folliculogenesis in females[8]. It has been used for more than forty years as a prescribed drug in neonatal medicine, mostly for apnoea treatment, but the overall effect of caffeine exposure at this age is poorly understood[9]. This study aimed to check the possible effects caffeine has on the pituitary ovarian axis.

METHODS

Animals

Eighteen (18) matured female *Sprague-Dawley* rats, weighing 140 g to 180 g were used for this study. The rats were purchased from The Animal House of the College of Medicine, University of Lagos. All procedures employed in this study were approved by the Institutional Experimentation and Ethics Committee (CM/HREC/010/16/064) of the College of Medicine, University of Lagos and are in conformity with International ethical standards on biomedical experimentation and animal care. The rats were housed in plastic rat cages in a well-ventilated animal room at room temperature at 12 hour's light and 12 hour's dark cycle. They were fed with standard rat chow and clean tap water *ad libitum*. The rats were left to acclimatize in the environment for two weeks before further studies commenced.

The rats were randomly divided into three groups (six rats per group) namely; Group-1 (Control); Group-2 (5 mg Caffeine administered rats); Group-3 (10 mg Caffeine administered rats) using GPower3.1 sample determination software. Caffeine was administered orally with the aid of an oral cannula at doses of 5 mg/kg and 10 mg/kg body weight daily for four weeks. Body weights of all experimental rats were taken at three days' interval throughout the period of the experiment. All caffeine treated rats were dosed the appropriate doses orally for four weeks after which they were fasted overnight and humanely sacrificed following the determination of their oestrous cycle phase using the vaginal lavage technique. Sacrifice was by cervical dislocation after CO_2 asphyxiation at oestrous and dioestrus phase respectively.

Determination of ovary weight and the corpus luteum number at oestrous and dioestrus phases of the oestrous cycle

The ovaries were carefully dissected free of fat under ice after sacrifice. The rats' ovaries were carefully dissected over ice and corpus luteum counted.

Serum TSH, FSH, and LH assay

Blood sample (5ml) was collected from the rat's retro-orbital sinus into plain sterile bottles, centrifuged at 556g for 15 min, and the serum was decanted from the plain bottle into Eppendorf' bottles for the assessment of anterior pituitary gland hormones (FSH, LH and TSH). All animals were fasted overnight before they were humanely sacrificed, and blood samples collected. Prior to commencement of the assay, all serum samples and reagents were brought to room temperature of 20-27 ºC. The microplate was formatted for the control, and the serum samples to be assayed in duplicate. 50µl of

appropriate serum reference, control, and rat's serum were pipetted into the appropriate well and 100 µl each of FSH-enzyme reagent solution was added to all wells. The microplate was swirled for 20-30 second to mix, after which it was covered and incubated for 60 min at room temperature. The content was discarded by decantation and the plate was blotted dry with absorbent paper. 350 µl of wash buffer was added and was repeated 3 times.100µl of working substrate solution was added to all the wells and was incubated at room temperature for 15 min. Then 50 µl of stop solution was added to each well and gently mixed for 15-20 second. The absorbance was read in each well at 450 nm (using reference wavelength of 620-630 nm to minimize well imperfections) in the microplate reader.

Statistical analysis

Data was analyzed with SPSS 15.0 for Windows Evaluation Version software using one-way analysis of variance (ANOVA). Results were presented as mean ± SEM, and the difference was considered significant at $p < 0.05$. Bar charts were utilized for graphical presentation of data.

RESULTS

Effect of caffeine on ovary weight of pubertal rats

The 5mg caffeine fed rats recorded a significant increase in the ovary weight (Figure 1, plate A) during the diestrous phase. There was a significant decrease in the ovarian weight during the diestrous phase 10mg group (Figure 1, plate A).

Corpus luteum count in caffeine-fed pubertal rats

During the oestrous and dioestrus phases, there was a significant increase in the corpus luteum count in both 5 mg and 10 mg groups (Figure 1, plate B).

Serum FSH, LH and TSH levels in caffeine exposed pubertal rats

Serum FSH levels increased significantly in both phases in the 10 mg group compared to the control. The 5mg group recorded a significant increase in the dioestrus phases (Figure 1, plate C). The LH of the 5 mg group was significantly increased during the oestrous phase (Figure 1, plate D). The 10 mg recorded a significant increase in serum LH levels. The 5 mg group recorded a significant increase in the TSH levels compared to the control (Figure 1, plate E).

DISCUSSION

The increasing report of reproductive disorders especially infertility among couples calls for concern especially in the light of reported increase in environmental pollution and lifestyle changes that promotes metabolic disorders[1]. Caffeine is a naturally occurring alkaloid found in the seeds, leaves and fruits of more than sixty plants such as coffee, cocoa kola nuts, tea, several other varieties of beverages including some carbonated drinks and some prescription drugs[10, 11]. While there was a significant increase in ovary weight in 5 mg caffeine-fed group at dioestrus, no significant change in the ovary weight was recorded during oestrous phase in the 5 mg/kg and the 10 mg/kg groups when compared with the control group. Other studies have reported a significant decrease in the weight of ovaries when caffeine was administered at high dose[12]. The decline in ovarian weight, following a higher dosage (10 mg/kg) of caffeine in the dioestrous phase, not only suggests an adverse effect on ovarian weight, but also indicates that its effect on reproductive function may be dose-dependent. Previous reports suggested that the methylxanthine owes the mechanism of its adverse effect to its inhibitory action on the growth of the primordial follicles which are a major contributory factor to ovarian weight[12]. This may lower female fertility which depends on the proliferation and maturation of ovarian cells[13].

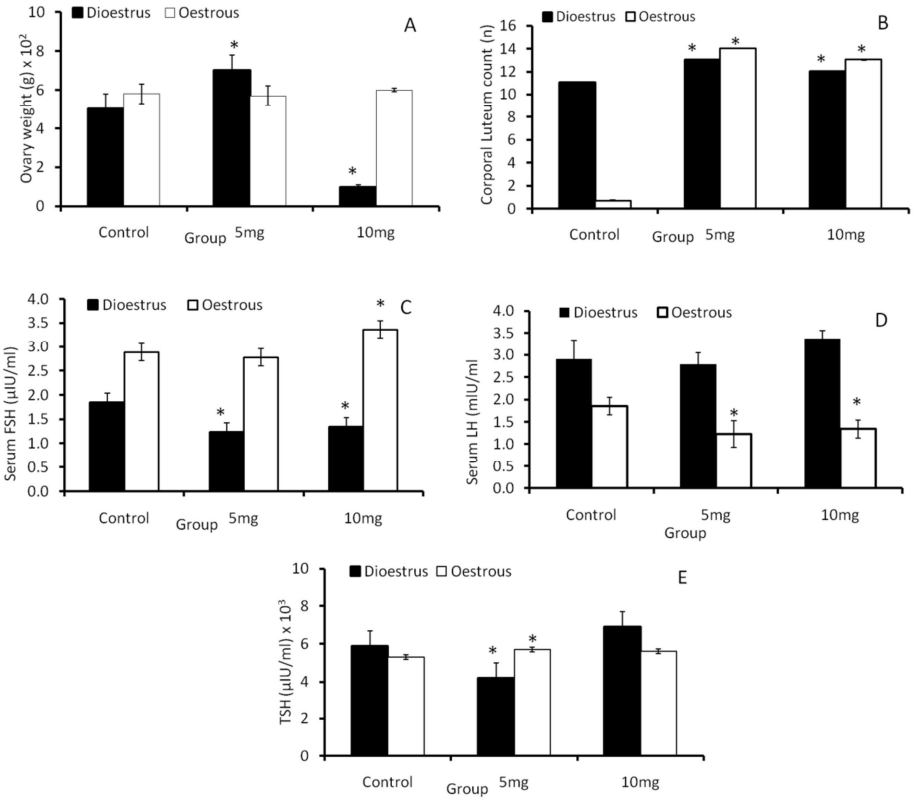

Figure 1: Effect of caffeine on ovary weight (plate A), corporal luteum count (plate B), serum FSH (plate C), serum LH (plate D), serum TSH (plate E) in pubertal female rats at different phases of oestrous cycle compared with control
* Significant difference ($p < 0.05$) compared to control at same phase of the oestrous cycle
n = 6, result displayed as mean ± SEM

The absence of any significant change recorded in the oestrous phase in both groups, as opposed to that of the 5 mg/kg group in the dioestrous suggests that caffeine impact on ovarian weight may be stage dependent in line with reports of previous studies[14]. A probable mechanism mediating reduction in ovarian may be due to its interference with cell division resulting in cell apoptosis and decreased number of cells[12]. Axelrod and Reichenthal[15] observed that caffeine precipitated genetic damage by interfering with cell faithful replication of its DNA[15]. Considering that female puberty is heralded by increased ovary weight[16], the decrease in ovary weight following the administration of caffeine suggests that caffeine, at even relatively high doses, may decrease ovarian growth.

This study demonstrated a significant (P < 0.05) increase in the corporal luteal count at the dioestrus phase between the control group when compared to the 5 mg caffeine group. There was a significant (P < 0.05) increase between the control group compared to the 10 mg caffeine treated group. There was a significant (P < 0.05) decrease between the 5 mg caffeine group and 10 mg caffeine group. These findings are in agreement with the previously reported increase in corporal luteal count[17]. According to Caan *et al.,* 1998[17], women who drank more than half a cup of tea per day had a significant increase in fertility which is due to the stimulatory effect of caffeine on corporal luteal. There was a significant (P < 0.05) increase in the corpus luteum count of the control group at oestrous phase when

compared to the 5 mg caffeine group. There was a significant ($P < 0.05$) increase between the control group and the 10mg caffeine group. However, Dorostghoal *et al.*,[12] recorded no statistically significant difference in the number of corpora lutea between the groups. Further analysis showed no significant ($P < 0.05$) decrease between the 5 mg caffeine group when compared to the 10 mg caffeine group. Studies have shown that throughout the oestrous cycle in rats, the highest oestrogen levels are during the pro-oestrus phase and these levels then drop during the oestrous phase and dioestrus phase respectively[18]. Coffee drinking may be associated with increased sex hormones binding globulin (SHBG). Coffee has been consistently associated with higher (SHBG) levels in women[19].

The present study at the dioestrus phase shows that there was no significant decrease ($P < 0.05$) between serum level of follicular stimulating hormone of the control group when compared to 5 mg caffeine group. There was a significant ($P < 0.05$) decrease between the control group when compared to 10mg caffeine-treated group in the dioestrous phase. This finding is in line with that of Oluwole *et al.,* 2016[20], who also recorded a significant decrease in FSH levels[20]. The fall in FSH levels in the study may be attributed to negative-feedback effect of ovarian hormones on the pituitary-ovarian axis. Fall in FSH in ovarian cell proliferation and maturation, resulting in decreased weight of ovary[21]. There was a significant decrease between the 5mg caffeine-treated group and the 10 mg caffeine-treated group. At the oestrous phase, this study also showed that there was a significant ($P<0.05$) decrease between the control group when compared to the 5 mg caffeine group. However, there was also a significant ($P<0.05$) increase between the control group when compared to the 10 mg caffeine group. The 5 mg caffeine group when compared to the 10 mg showed a significant increase. Mechanism mediating this is increase in the oestrous phase is subject to further studies.

A variety of pathophysiologic effects of caffeine components of caffeinated beverages on sex hormones and ovulatory function exist. Studies in animal models give an indication that caffeine can inhibit oocyte maturation or enhance steroid production via inhibition of phosphodiesterase[8,22]. Caffeine may have a positive, inverse or null association with E2 but has no effect on ovulatory function although no studies till date have prospectively measured caffeine intake at multiple time points and directly measured ovulation[23, 24, 25].

Thyroid stimulating hormone showed a significance ($P<0.05$) increase between the control group when compared to the 5mg caffeine group. Comparing the control group and the 10 mg caffeine group there was a significant ($P < 0.05$) increase. Comparing the 5 mg caffeine group and the 10 mg caffeine group there was no significance ($P > 0.05$) dioestrus phase. At the oestrous phase there was significant ($P > 0.05$) decrease between the control group and the 5mg caffeine group. This biphasic response could be due to the direct effect of caffeine on the thyroid gland[25]. Comparing the control group and the 10 mg caffeine-control group there was a significant ($P < 0.05$) increase in the 5mg caffeine-control group and the 10 mg caffeine-control group showed significant ($P < 0.05$) increase. Caffeine administration has been known to increase basal serum TSH levels[26]. This effect of caffeine may result in elevation of cyclic adenosine monophosphate (cAMP) concentration which is necessary for growth and secretion of thyroid follicles. Caffeine, a phosphodiesterase inhibitor, has a stimulatory effect on intracellular cAMP production[27] and also prevents the destruction of cAMP by cAMP phosphodiesterase inhibition[28]. cAMP functions as a second messenger and mediates all the intercellular effects of TSH[27]. Another possible mechanism on the stimulation of an increase in TSH production is the increasing effect on ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in thyroid, which stimulates the growth of

follicular cells hence leading to an increase in TSH[29]. Son *et al*., 2003 recorded that caffeine increased the weight of pituitary gland which in turn increased the activity of the thyroid gland and further led to an increase in TSH secretion[30]. Luteinizing hormone levels in serum showed significant decrease when compared to the control group and the 5mg caffeine-control group. This finding is in line with that of Oluwole *et al*., 2016[20], who also recorded a significant decrease in LH levels. The control group and the 10 mg caffeine-control group showed a significant increase. Comparing the 5 mg caffeine group and the 10 mg caffeine-control group showed significant increase at the dioestrous phase.

At the oestrous phase there was a significant decrease between the control group and the 5 mg caffeine-group. The control group and the 10 mg caffeine-group showed a significance decrease. Comparing the 5 mg caffeine-group and the 10 mg caffeine-group there was a significant increase.

CONCLUSION

It can be concluded from the result of this study that caffeine promotes growth of the follicles with resultant increase in the number of corpus luteum indicating its stimulating effect on FSH and LH of the pituitary-ovarian axis.

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