INVESTIGATION OF THE EFFECTS OF ALLOXAN-INDUCED DIABETES ON REPRODUCTIVE HORMONES (FOLLICLE STIMULATING, LUTEINIZING AND PROLACTIN), LIPID PROFILE AND SERUM ELECTROLYTES IN MALE AND FEMALE WISTAR RATS

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ABSTRACT

Human patients with Type1 diabetes and inadequate metabolic control have been found to exhibit a high prevalence of infertility but the exact mechanism by which this happens has not been fully elucidated. Alterations in the levels of lipids and electrolytes are considered to be good indications of some certain diseases including diabetes. This study aims at elucidating the impact of diabetes on the reproductive functions of male and female subjects as well as the responses of lipid profile which could be a determining factor affecting diabetic condition, to Alloxan-induced diabetes using male and female Wistar rats. To explore this, various hormonal assays assessing reproductive hormones such as Follicle stimulating hormone (FSH); Luteinizing hormone (LH); Prolactin hormone (PRL); lipid profile analyses and electrolytes measurements were employed. Our results showed that diabetes induce significant reduction in the levels of FSH and LH in both male and female rats ($p<0.0001$); significant reduction in the levels of LH in male ($p<0.01$) and female ($p<0.0001$); significant increase in the levels of prolactin (PRL) in male ($p<0.05$) and female ($p<0.0001$) ; significant increases in cholesterol levels in both male and female rats ($p<0.01$), *an indication that diabetes is accompanied in most cases by hypercholesterolemia* and triglycerides levels in both male ($p<0.01$) and female ($p<0.0005$) rats when compared to the control rats. The results obtained in this study shed more light on the mechanism by which fertility in male and female subjects is affected by diabetes and suggest possible mechanisms by which infertility could result from diabetes.

Key words: Diabetes, hormones, prolactin, Follicle-stimulating, Luteinizing, cholesterol.

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INTRODUCTION

Diabetes is a chronic, metabolic disease that is characterized by elevated levels of blood glucose which over time results in serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or does not make enough insulin. The prevalence of type 2 diabetes has risen dramatically in the past thirty years in more developed countries. A globally agreed target to halt the rise in diabetes by 2025 is now the major goal of the world at large. (WHO,2016).Type I diabetes mellitus in experimental animals has been widely induced by employing Alloxan, a diabetogenic agent of molecular weight and formula of 160.07g/mol and $C_4H_2N_2O_4$ respectively (Viana *et al.,* 2004). Alloxan exhibits selective toxicity to pancreatic beta cells as it preferentially accumulates in the beta cells as glucose analogues (Rohilla and Ali, 2012).

Many reports have focused on the link between type 1diabetes and fertility but the mechanisms by which diabetes affect the fertility status in both animals and humans are still under intensive study. Human patients with Type1 diabetes and inadequate metabolic control have been found to exhibit a high prevalence of infertility (Codner *et al*., 2012).Systemic effects such as disruptions in the hypothalamic pituitary gonadal axis which may consequently contribute to a loss in fertility have

been attributed to diabetes. Under normal situation, the secretion of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary is being stimulated by GnRH pulses released by the hypothalamus of hypothalamic pituitary gonadal axis. The actions of LH on Sertoli cells as well as that of FSH on the Leydig cells have been reported to be involved in the activation of the process of spermatogenesis (Schoeller *et al*., 2012). Alterations in male reproductive system have been widely reported in individuals with diabetes but there are fewer studies regarding sexual dysfunction among diabetic women (Enzlin *et al*., 2002; Erol *et al*., 2002; Erol *et al*., 2003; Omidvar *et al*., 2013. Testosterone, an important hormone involved in male reproductive system has been reported to be initiated by stimulation of LH by the binding of hormone to LH receptors on Leydig cells through adenylate cyclase. A recent study has also pointed to the fact that low level of FSH was associated with prediabetes and diabetes in postmenopausal women but the underlining principles behind these associations has been based suggestively on the adiposity and insulin resistance (Wang *et al*., 2016) while the cellular mechanism behind insulin resistance is not fully understood. Reports have it that diabetic men exhibit up to 50% sexual dysfunction prevalence while this is lower in diabetic women (Enzlin, *et al*., 2002; Jackson, 2004. In diabetic males, impotence, retrograde ejaculations, decreased sperm motility and concentration, poor semen quality as well as decreased fertility potential are conditions that have been described (Ramalho-Santos *et al.,* 2008). Prolactin (PRL) has been recognized as a hormone involved in lactogenic activity since early 1970 (Wass and Stewart, 2011). Prolactin levels have been found to increase in people with fasting hyperglycemia and specifically in women, it has also been reported that even a mild increase in the level of prolactin can cause infertility irrespective of normal menstrual cycle. Loss of libido, erectile dysfunction and infertility has been reported to result from hyperprolactinemia in men (Epstein, 2011). Decrease in bone mineral density has also been linked to high level of prolactin. The glucose metabolic regulation effect of prolactin has been reported not to be confined to the period of pregnancy, (Terra *et al.,* 2011; Labriola *et al*., 2007) however the link between circulating prolactin and glucose metabolism outside pregnancy has not been elucidated in epidemiological studies(*Wang et al*., 2013). Due to many conflicting ideas relating diabetes to high prolactin level, it is important to note that elevated serum prolactin levels usually found in diabetics may not be an actual cause of hyperprolactinemia.

Insulin that is produced normally in the body escorts glucose which is consequently converted to energy in the body. In the same way, triglycerides also act as source of energy with the help of insulin helping the body to do this. High level of triglycerides in the body has been identified to be caused by excess carbohydrates in the diet (Parks, 2001) and insulin resistance is signaled by high triglycerides level resulting in higher than normal blood sugar levels. Triglycerides also stimulate the build-up of fat and cholesterol in the blood vessels. Untreated high blood cholesterol can result in atherosclerosis which is a condition of build-up of plaque in the blood vessels which may consequently increases the risk of heart attack, stroke, and peripheral artery disease (Ass, 2003); Varbo *et al.,* 2013). Dyslipidemias has been observed in many people with diabetes and they have a combination of low "good" cholesterol, and high "bad" cholesterol. The body's electrolyte control system has also been reported to be broken down whenever diabetes disrupts metabolic function (Wang *et al.,* 2013) but the link between this and fertility has not been fully elucidated.

Considering all the aforementioned, our study therefore was designed to explore all the possible mechanisms by which diabetes influence fertility, lipid profile and electrolytes balance in experimental male and female Wistar rats.

Materials and Methods

Forty (40) healthy male and female Wistar rats - Kyoto strain (20 females and 20 males) weighing between 100-160g were obtained from the Federal University of Agriculture, Abeokuta, Nigeria. The Wister rats were housed in metabolic cages and maintained in well ventilated room provided with 12:12 h light and dark cycle for each 24h cycle at a temperature of approximately 25°C and were fed on pellets feeds (obtained from Animal care store, Ibadan, Oyo State, Nigeria) and tap water ad libitum. After the initial period of seven days acclimatization, the rats were grouped into four

groups containing ten (10) rats each based on weight and sexes as below. The rats were treated according to Guidelines on care and use of laboratory animals from Hopkins University. Group A (Normal control Male) and Group C (Normal control Female) were administered normal saline intra-peritoneally while Group B (Diabetic Male) and Group D (Diabetic Female) were injected with Alloxan ($C_4H_4N_2O_2$) (Sigma chemical company, St. Louis, U.S.A) dissolved in saline buffer (0.1N, pH 4.0) at a dose of 130mg per kg body weight to induce diabetes. The Wistar rats were fed ad libitum and maintained for the period of the experiment. Accu-Chek Active glucometer (serial no GC04652832), a product of Roche Diagnostic GmbH, Germany was used to establish diabetic state by demonstrating a two-three-fold increase in their blood glucose (hyperglycemia) compared with the normal control group one week after induction. After two weeks of induction of diabetes, blood was collected from the inferior *vena cava* of heart of the animals using sterile 5 ml needle and syringe into plain centrifuge tubes and was allowed to stand for 1 hr. Serum was prepared by centrifugation at 3000 ×g for 15 minutes in a centrifuge. The clear supernatant was used for analysis. Lipid profile (assessing the levels of triglycerides and total cholesterol) and hormonal assays assessing the levels of Follicle stimulating, progesterone, prolactin and Luteinizing hormones were then determined. Concentrations of FSH, LH and prolactin in serum samples were determined in both diabetic and control groups using Enzyme Immunoassay (EIA) technique with Enzyme-Linked Immunosorbent Assay (ELISA) Kit, a product of Sigma chemical company, St. Louis, U.S.A according to manufacturer's instructions. Total cholesterol in the blood samples was assessed by the method involving enzymatic hydrolysis and oxidation using Cell Biolabs' Total Cholesterol Assay Kit, a product of Cell Biolabs INC., San Diego, USA according to manufacturer's instructions. Triglycerides level was assessed by a colorimetric method involving hydrolysis with lipases using serum triglycerides quantification kit,a product of Cell Biolabs INC., San Diego, USA according to manufacturer's instructions. Serum calcium and serum chloride were determined by reagent diagnostic kit, a product of Randox Laboratories Ltd, UK according to manufacturer's instructions.

Serum sodium was assayed using a colorimetric method based on modifications of those first described by Maruna,1958 and Trinder, 1951 in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen. Sodium reagent colorimetric kit, a product of TECO Diagnostics, California, U.S.A was employed in this study.

Statistical Analysis

All data are expressed as mean ± SEM. Statistical analyses between diabetic groups and the control groups were carried out using one-way analyses of variance ANOVA followed by Turkey ad hoc test. Values for $p<0.05$ are considered to be statistically significant. Graphpad prism 7 software was employed for these analyses.

Results

Diabetes was significantly induced in both experimental male and female Wistar rats when compared to their corresponding control groups after one week of single intraperitoneal injection of 130mg/kg Alloxan as presented in Figure1. Significant reductions in the concentration of FSH were observed in both diabetic male ($p<0.0005$) and female ($p<0.0001$) rats when compared to their corresponding controls (Figure 2). Also, significant reductions in the concentration of LH were observed in both diabetic male ($p<0.01$) and female ($p<0.0001$) rats when compared to their corresponding controls (Figure 3). Significant increases in the concentration of prolactin hormone were observed in both diabetic male ($p<0.05$) and female ($p<0.0001$) rats when compared to their corresponding controls (Figure 4).

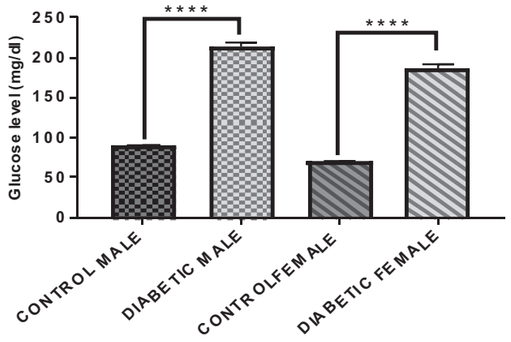

Figure 1: Induction of diabetes in experimental Wistar rats
Error bars indicate standard error of mean obtained from theglucose levels measurements. Four asterisks indicate p<0.0001.

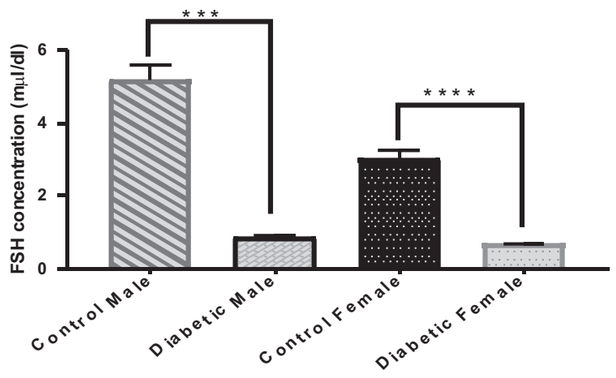

Figure2: Effect of Alloxan-induced diabetes on the level of Follicle Stimulating Hormone (FSH)
Error bars indicate standard error of mean obtained from the FSH levels measurements. Three asterisks indicate p<0.0005 and four asterisks p<0.0001.

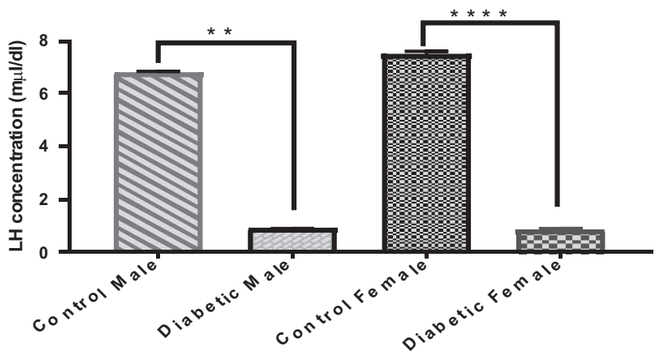

Figure 3: Effect of Alloxan-induced diabetes on the concentration of Luteinizing Hormone (LH)
Error bars indicate standard error of mean obtained from the LH concentration assessment. Two asterisks indicate p<0.01 and four asterisks p<0.0001.

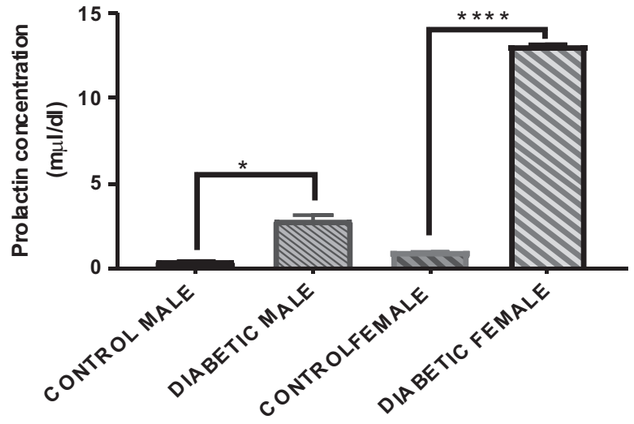

Figure 4: Effect of Alloxan-induced diabetes on the concentration of Prolactin Hormone (PRL)
Error bars indicate standard error of mean obtained from the prolactin hormone concentration assessment. One asterisk indicates p<0.05 and four asterisks p<0.0001.

The results of the lipid profile showed, significant increases (p<0.01) in the level of total cholesterol in both diabetic male and female rats when compared to their corresponding controls, likewise significant increases in the level of triglycerides in both diabetic male (p<0.01) and female (p<0.0005) rats when compared to their corresponding controls as presented in Figures 5 and 6.

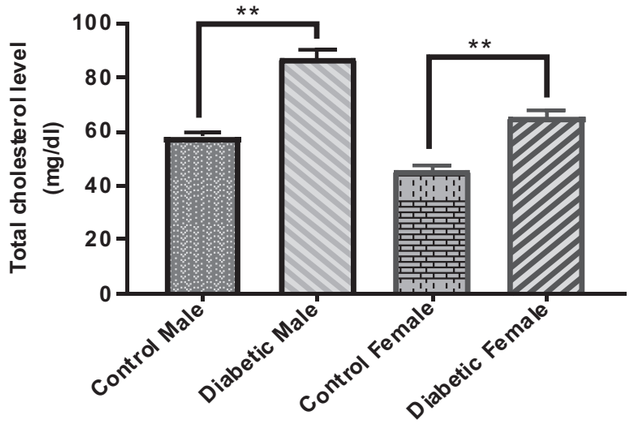

Figure 5: Effect of Alloxan-induced diabetes on total cholesterol level
Error bars indicate standard error of mean obtained from the total cholesterol. Two asterisks indicate p<0.01.

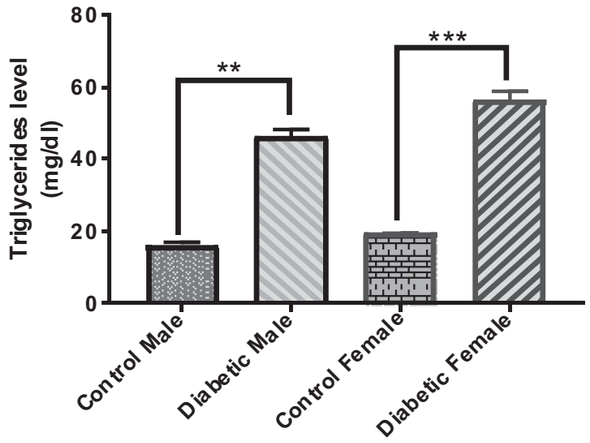

Figure 6: Effect of Alloxan-induced diabetes on triglycerides level
Error bars indicate standard error of mean obtained from the triglycerides measurement. Two asterisks indicate $p<0.01$ and three asterisks indicate $p<0.0005$.

The effects of diabetes on Serum electrolytes, showed that there were significant increases in the level of serum calcium, chloride and sodium in male ($p<0.01$) while in the female, significant increase in calcium ($p<0.05$) level was noted but the difference in the chloride and sodium level was not significant when compared to their corresponding controls(Figures 7, 8 and 9).

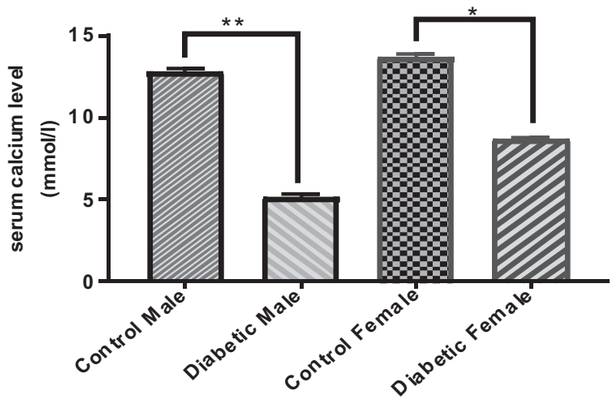

Figure 7: Effect of Alloxan-induced diabetes on serum calcium level
Error bars indicate standard error of mean obtained from serum calcium measurement. Two asterisks indicate $p<0.01$ and one asterisk indicate $p<0.05$

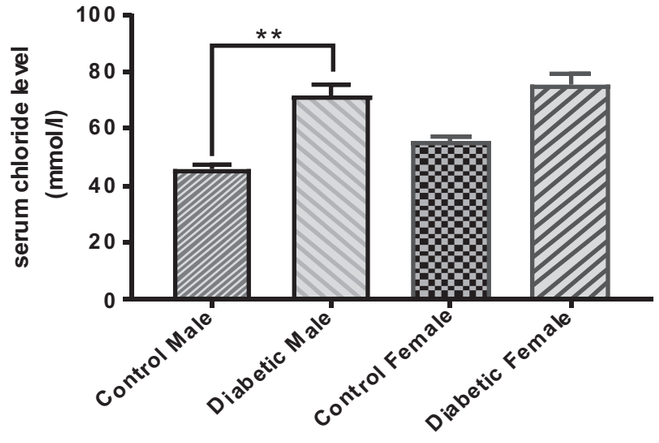

Figure 8: Effect of Alloxan-induced diabetes on serum chloride level
Error bars indicate standard error of mean obtained from serum chloride measurement. Two asterisks indicate $p<0.01$.

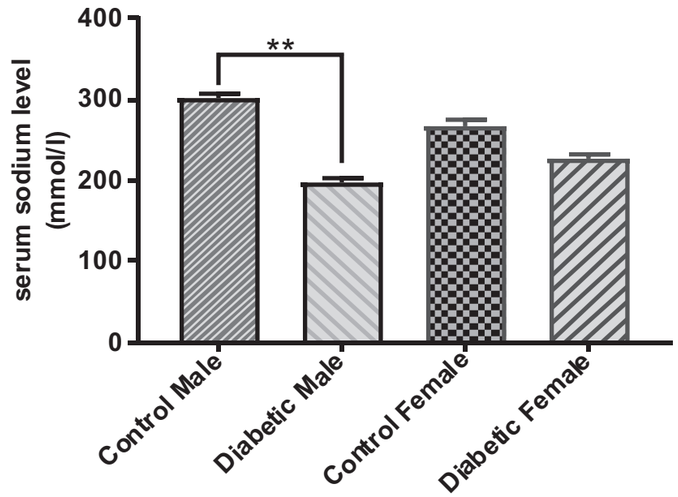

Figure 9: Effect of Alloxan-induced diabetes on serum sodium level
Error bars indicate standard error of mean obtained from serum sodium measurement. Two asterisks indicate $p<0.01$.

DISCUSSION AND CONCLUSION

Diabetes Mellitus has been recognized as a clinical syndrome since ancient times and remain a crippling global health problem till today (Wild *et al.,* 2004; Olokoba *et al.,* 2012). Diabetes in relation to reproductive functions in men remains a global key focus being investigated effortlessly in order to overcome the problem of infertility in humans. The results of this study showed significant reductions in FSH and LH levels in male Wistar rats following diabetic induction with Alloxan. These observed reductions corroborate previous work on male human subjects (Adegbesan and Ogunlabi, 2013), thus confirming that diabetic condition affects reproductive activities negatively in both human and animal male subjects.Ballester *et al*., 2004also reported that an insulin-dependent reduction in the concentration of FSH results in concomitant reduction in LH levels, thus supporting the results of this study that reduction in FSH level subsequently influenced similar effect on LH level. These results suggest that reductions in the levels of FSH and LH may affect factors affecting reproductive functions and consequently resulting in infertility.

The results on serum prolactin level assessment indicated significant increase (at $p<0.01$) in the serum level of diabetic female rats. This result is in line with the findings ofMooradian, 1985. The association between diabetes and serum PRL levels has been considered to be positive in some studies while some other studies have shown an inverse association between serum PRL levels and diabetes (Wang *et al*., 2013; Balbach, 2013). The results obtained in this study in male Wistar rats indicate a significant increase in the level of prolactin in male diabetic rats (at $p<0.05$). Another observation about this prolactin result is that the level of prolactin in males is significantly reduced to what was observed in their female counterparts suggesting that prolactin hormone responsible for lactation in female is more relevant in females than males. This result is buttressed by the findings of Lambert *et al.,* 2013 who found out that gender factor is a significant predictor of prolactin levels.

The observed significant increases ($p<0.01$)in the levels of cholesterol in Alloxan-induced diabetic male and female Wistar rats, is an indication that diabetes is accompanied in most cases by hypercholesterolemia. Similarly, Bako *et al.,* (2014); Abdulazeez *et al.,* (2017) observed significant increase in cholesterol concentrations in Alloxan-induced diabetic rats when compared to the normal control rats. The results of triglycerides analysis also showed significant increases in male diabetic rats ($p<0.01$) and in female diabetic rats ($p<0.0005$) when compared to their corresponding control groups. This finding is in agreement with the report of Bako *et al*., (2014) and *A*bdulazeez *et al.,* (2017). *E*levated cholesterol and triglycerides levels have been attributed to coronary heart disease (Varbo *et al*., 2013).In addition, significant increases in serum cholesterol levels and triglyceride contents in the diabetic group than in the control group, revealed the typical lipid profile of diabetes (Simonen *et al*., 2002). The observed significant reduction observed in the level of LH and elevations in lipid profiles of female diabetic rats provide a form of link between diabetes and fertility. This is buttressed by the finding of Zachurzok *et al*., 2016 who reported that Lipid profile in diabetic adolescent girls is adversely influenced by the androgens level. Since LH is responsible for the synthesis of androgen such as testosterone and other hormones that influence the growth and development of the male reproductive system, then the observed significant reduction in the LH concentration in male diabetic rats and significant elevation of lipid profile when compared to the control group suggests infertility response due to diabetes.

Previous studies have established a correlation between disturbances in the levels of some electrolytes and diabetes mellitus (DM) (Al-Rubeaan *et al*., 2011; Katz, 1973).Our results on the effect of diabetes on serum sodium revealed significant reduction in the male diabetic rats (at $p<0.01$) when compared to the normal control group. This result is supported by the work of Wang *et al*., 2013 that revealed that Serum sodium and magnesium levels were decreased in Chinese subjects with diabetes (Wang *et al*., 2013). Also, observed significant reductions in the levels of serum calcium in both diabetic male and female rats at $p<0.01$ in this study suggest reduction in bone mass which has been reported to be attributed to poor blood glucose control. This result is

supported by the findings of Hassan *et al*., 2016; Thalassinos *etal*., 1993 who reported that increased urinary calcium excretion is observed during poor blood glucose control and that reduced bone mass in diabetes mellitus may have resulted from this. Conclusively, our study revealed the importance, possible mechanism and link of diabetes status to fluctuations in reproductive hormonal status, lipid profile and electrolytes. This study also suggests that observed alterations in hormonal status, lipids and electrolytes levels in diabetic state may serve as potential risk factors for many other diseases including infertility.

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