

Sugar Intake Disrupts some Reproductive Functions in Female Wistar Rats

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Received: 15/10/2020

Accepted: 08/01/2021

Published: 20/03/2021

Abstract

The increasing rate of infertility has raised a lot of concern. Studies show that lifestyle and dietary factors affect reproductive functions. Sugar, a universal sweetener in the diet has been linked with metabolic syndrome and disruption of male reproductive functions. The objective of the current study is the assessment of the effects of sugar on some reproductive functions of female Wistar rats. Twenty (20) adult female Wistar rats (180-200 g) randomly divided into four (n=5), received 10 mL/Kg distilled water (group 1 - control), 6.25, 20, and 64 mg/Kg Sugar (groups 1, 2, 3) daily by oral gavage for three (3) weeks, respectively. Parameters evaluated were fasting blood glucose, estrous cycle, histology and relative weights of reproductive organs, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol hormone levels. Sugar significantly decreased ($p < 0.05$) fasting blood glucose level and relative weights of ovary and uterus compared to control. It also significantly reduced the frequency of estrus and diestrus phases but caused significant increases ($p < 0.05$) in the metestrus phase of the estrous cycle and FSH level in comparison with the control. There were anomalies in the histology of the ovary and uterus. Therefore sugar disrupted the estrous cycle, altered the weights and cytoarchitecture of the ovary and uterus. Sugar consumption has harmful effects on some reproductive functions of female Wistar rats.

Keywords: Rats, Ovary, Sugars, Estrous cycle, Infertility

Introduction

The increasing rate of infertility has raised a lot of concern, according to (1) infertility affects 48.5 million people of reproductive age globally. Infertility occurs in both male and female partners, while male infertility resulted from low sperm count/abnormal sperm function caused by genetic defects/DNA damage, varicocele, and premature ejaculation among others (2), that of women resulted from hormonal disorders, blocked fallopian tubes and endometriosis among others (3). Besides genetic predisposition, lifestyle factors such as obesity, malnutrition, reduced exercise, smoking, and psychological stress adversely alter several aspects of female reproductive health, which includes ovarian function, fertilization, implantation, and embryo development (4, 5). Weight loss has also been associated with a reduced frequency of ovulation/cessation of ovulation (6). Furthermore, dietary lifestyle is becoming popularly associated with reproductive health. A common and widely consumed food substance is sugar (sucrose), added to diets to improve its palatability. Sugar comes from naturally occurring sources such as fruits, vegetables, milk, and dairy foods. Sugar is also produced commercially as refined sugar at sugar mills, and sugar refineries (7). The world sugar production amounted to about 175.1 million metric tons in 2014/2015 (8). The growth of the global production of sugar led to an increase in real consumption of sugar in the world (9), though the American Heart Association (AHA) had recommended an average sugar consumption of 150 calories/day (10).

Sugar primarily gives energy to the brain and nervous system to regulate the performance of day-to-day activities (11). It is a universal sweetener for a variety of food products such as beverages, sweets, bakery, and dairy products among so many others (12). The role of sugar in the diet and its relationship to health has become a course of concern as evidence links it to a variety of health problems (13). Sugar-sweetened beverages were linked with tooth decay and metabolic syndrome, while refined sugar altered sperm quality and male reproductive function (14, 15) reported that high sucrose and high salt diet combination promoted weight loss. (16) reported that sugary drinks reduced sperm motility in healthy men (17, 18) while a diet high in sucrose reduced testosterone level which hampered spermatogenesis, mediating alteration in sperm quality (17, 19), necessitating sugar consumption regulation in the diet to less than 10% of total energy intake (20). However, there is a dearth of information correlating female reproductive functions and sugar intake. Hence this study assessed the effects of sugar intake on some reproductive functions in female Wistar rats.

Materials and methods

Experimental design

All protocols involving the use of animals were in line with the Bingham University Animal Care and Use guideline, and the study

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conformed to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources for the National Research Council. Twenty (20) female Wistar rats (180-200 g) obtained from the National Veterinary Research Institute Plateau State Nigeria, were kept under standard laboratory condition and acclimatized for two (2) weeks, with access to feed and drinking water ad libitum before commencing administration. There were four groups of rats (n=5) that received 10 mL/Kg distilled water (group 1 - control), 6.25, 20, and 64 mg/Kg Sugar (groups 1, 2, 3) daily by oral gavage for three (3) weeks, respectively. A mean estrous cycle length of 4 days was the basis for the administration period as reported by Long and Evans (1922), providing an opportunity to study the effect of sugar on the rat in 5 consecutive estrous cycles. Dangote Refined Granulated White Sugar® (Dangote Sugar Refinery PLC, Nigeria) was freshly prepared by dissolving it in distilled water daily before use. The dosage regime used was in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline (21). Recording of the body weights was weekly and just before sacrifice with an electronic weighing scale (EK5055, China).

Assessment of the estrous cycle

This was as described by (22), done between the hours of 7:00 am - 8:00 am every morning for each rat for three weeks before treatment with sugar. Afterward, the control group received distilled water, while each experimental group received its designated dosage for another three weeks during which the estrous cycle was further assessed (23, 24). Viewing of cells was with a light microscope at 40× magnification.

Blood collection and serum preparation

Before euthanization, a drop of blood was gotten from the tail of the fasted rats for determination of the fasting blood glucose level with an automated glucometer (Fine test IGM-00178, UK). Rats were killed by thiopental anesthesia, before dissection along the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart and the organs. By a cardiac puncture, blood was gotten into plain serum bottles, and allowed to coagulate for at least 45 min and then centrifuged at 1764 g for 15 min, for easy aspiration of the supernatant (serum), stored at -20 °C.

Hormonal analysis

Follicle-stimulating hormone, luteinizing hormone, and estradiol levels assays were from the stored serum using enzyme-linked immunosorbent assay (ELISA) kits (Fortress Diagnostics, UK).

Collection of the organ (ovaries and uterus) and histological preparation

The ovaries and uterus freed from attached tissue were immediately weighed using a digital electronic scale (Camry EHA501, China), fixed in Bouin's fluid, and processed for microscopic examination. The process involves sectioning of the embedded tissue with a microtome to get the 4–5 µm-thick paraffin sections, dewaxing, hematoxylin, and eosin (H&E) staining and viewing of the slides with a light microscope at 40× magnification.

Statistical analysis

The data obtained were calculated using the Graph Pad Prism Statistics software (USA) version 5.0 and expressed as mean ± standard error of the mean (mean ± SEM). The level of significance was at $p < 0.05$.

Results

Effect of sugar on fasting blood glucose level

There were significant decreases ($p < 0.05$) in fasting blood glucose level of 6.25 mg/Kg sugar and 20 mg/Kg sugar treated groups compared to the control. Also, there was a significant decrease ($p < 0.05$) in the fasting blood glucose level of the 6.25 mg/Kg sugar treated group when compared to the 64 mg/Kg sugar treated group (Figure 1).

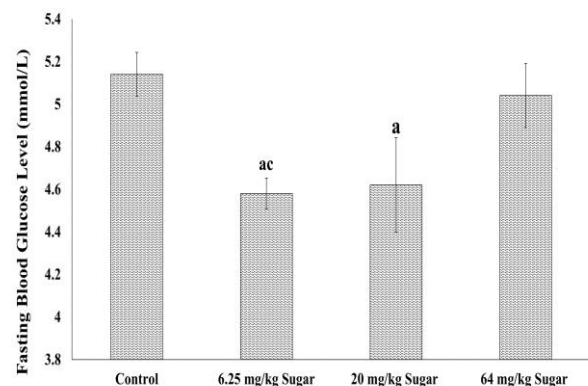


Figure 1. Effect of sugar (sucrose) on fasting blood glucose level. Columns represents mean ± standard error of mean, a= $p < 0.05$ compared to control, c= $p < 0.05$, n= 5

Effect of sugar on body weight gain

There were no significant differences in body weight gain of all the sugar-treated groups when compared to the control and other groups (Table 1).

Table 1. Effect of sugar on body weight gain

| Groups | Body weight gain (g) | | |
|------------------|----------------------|----------|----------|
| | Week 1 | Week 2 | Week 3 |
| Control | 1.4±0.24 | 2.0±0.32 | 2.2±0.24 |
| 6.25 mg/Kg Sugar | 1.4±0.22 | 1.5±0.21 | 1.6±0.22 |
| 20 mg/Kg Sugar | 1.6±0.24 | 1.8±0.49 | 1.8±0.49 |
| 64 mg/Kg Sugar | 1.4±0.24 | 1.6±0.68 | 2.2±0.37 |

Values expressed as mean ± standard error of mean, n=5

Effect of sugar on relative organ weight

The result showed a dose dependent significant decreases ($p < 0.05$) in the relative weight of the ovary and uterus of the 20 and 64 mg/Kg sugar treated groups when compared to the control. Also, there was a significant decrease ($p < 0.05$) in the relative weight of the ovary of the 64 mg/Kg sugar treated group compared to the 6.25 mg/Kg sugar treated group (Table 2).

Table 2. Effect of sugar on relative organ weight

| Groups | Relative ovary weight (%) | Relative uterus weight (%) |
|------------------|---------------------------|----------------------------|
| Control | 0.078±0.005 | 0.062±0.012 |
| 6.25 mg/Kg Sugar | 0.072±0.004 | 0.051±0.007 |
| 20 mg/Kg Sugar | 0.056±0.005 ^a | 0.037±0.011 ^a |
| 64 mg/Kg Sugar | 0.045±0.012 ^{ab} | 0.037±0.012 ^a |

Values expressed as mean ± standard error of mean. a = p < 0.05 compared to control, b = p < 0.05 compared to 6.25 mg/Kg sugar, n=5.

Effect of sugar on reproductive hormone level

The result shows a significant increase (p < 0.05) in the serum follicle-stimulating hormone level of the 64 mg/Kg sugar treated group relative to the control, 6.25, and 20 mg/Kg sugar groups. There were no significant differences (p < 0.05) in the serum levels of luteinizing hormone and estradiol (Figures 2 and 3).

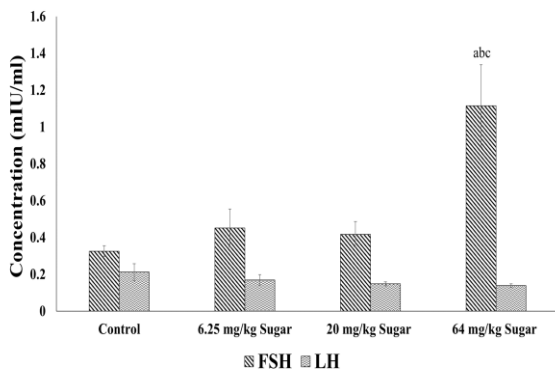


Figure 2. Effect of sugar on serum level of follicle stimulating hormone and luteinizing hormone. Columns represent mean ± standard error, a = p < 0.05 compared to control, b = p < 0.05 compared to 6.25 mg/Kg sugar, c = p < 0.05 20 mg/Kg sugar, n=5.

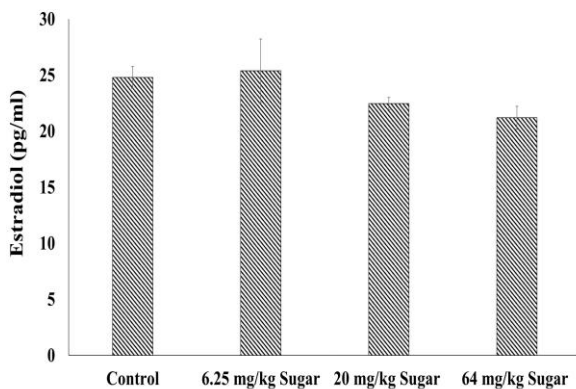


Figure 3. Effect of sugar on serum estradiol level. Columns represent mean ± standard error, n=5.

Effect of sugar on the vaginal epithelial cells

Figure 4, show the vaginal smear of the sugar treated groups in four phases of the estrous cycle with the right proportion of epithelial cells.

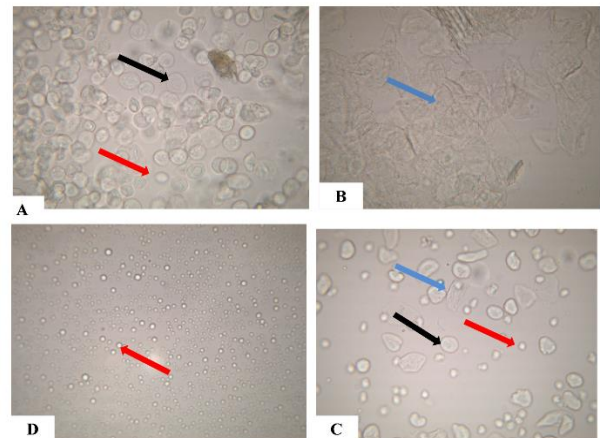


Figure 4. Photomicrograph of vaginal epithelial cells of Wistar rats given the sugar. (A) Proestrus phase (nucleated epithelial cells - black arrow and leukocytes - red arrow). (B) Estrus phase (anucleated cornified cells - blue arrow). (C) Metestrus phase (leukocytes - red arrow, cornified - blue arrow, and nucleated epithelial cells - black arrow). (D) Diestrus phase (leukocytes - red arrow). 40x magnification.

Effect of sugar on the estrous cycle

There were no significant differences (p < 0.05) in the lengths of the estrous cycle before and after treatment with sugar (table 3). While the 20 mg/Kg sugar caused a significant decrease (p < 0.05) in the frequency of the estrus phase, it concurrently caused a significant increase (p < 0.05) in the frequency of the diestrus phase when compared to the control and 6.25 mg/Kg sugar treated groups (Table 4). The 6.25 mg/Kg sugar treated group show a significant increase (p < 0.05) in the frequency of the metestrus phase when compared to the control and 64 mg/Kg sugar treated groups (Table 4).

Table 3. Effect of sugar on the length of estrous cycle (days)

| Groups | Estrous cycle length (days) | |
|------------------|-----------------------------|-----------|
| | Pre-treatment | Treatment |
| Control | 9.9±2.14 | 5.5±1.21 |
| 6.25 mg/Kg Sugar | 7.6±3.16 | 9.7±2.02a |
| 20 mg/Kg Sugar | 9.8±2.78 | 6.6±2.06 |
| 64 mg/Kg Sugar | 7.8±2.49 | 5.4±0.76b |

Values expressed as mean ± standard error, a = P< 0.05 compared to control, b = P< 0.05 n=5 compared to 6.25 mg/Kg Sugar.

Table 4. Effect of sugar on the estrous cycle

| Groups | Proestrus phase | | Estrus phase | | Metestrus phase | | Diestrus phase | |
|------------------|-----------------|------------|---------------|--------------------------|-----------------|--------------------------|----------------|--------------------------|
| | Pre-treatment | Treatment | Pre-treatment | Treatment | Pre-treatment | Treatment | Pre-treatment | Treatment |
| Control | 21.42±2.38 | 34.52±5.29 | 27.38±1.77 | 27.38±2.77 | 16.66±3.53 | 16.66±1.06 | 32.14±5.29 | 19.04±3.98 |
| 6.25 mg/Kg Sugar | 28.57±5.43 | 34.52±1.77 | 29.76±1.77 | 23.80±3.01 | 19.83±1.06 | 27.38±2.32 ^{ac} | 27.38±2.32 | 14.27±3.68 |
| 20 mg/Kg Sugar | 28.57±3.98 | 30.95±2.38 | 23.80±1.51 | 13.09±4.09 ^{ab} | 18.65±1.45 | 20.23±2.76 | 33.33±2.61 | 35.71±2.38 ^{ab} |
| 64 mg/Kg Sugar | 24.99±4.85 | 36.50±5.29 | 23.81±3.69 | 17.85±2.32 | 26.22±1.08 | 19.04±1.51 | 34.52±4.85 | 22.62±5.09 |

Values expressed as mean ± standard error, a = p < 0.05 compared to control, b = p < 0.05 compared to 6.25 mg/Kg sugar, n=5.

Effect of sugar on histology of the ovary and uterus

The ovarian section of 6.25 mg/Kg sugar showed atretic follicles and distorted stroma, that of 20 mg/Kg sugar showed vacuoles within stroma cells (figure 5). The 64 mg/Kg sugar cause direr effects including atretic follicles, distortion, and vacuolations in stroma cells as compared with the control (figure 5). The uterine lumen and epithelial lining of the sugar treated groups all appeared abnormal, the uterine section of 6.25 mg/Kg sugar further showed degenerated uterine gland as compared to the control (Figure 6).

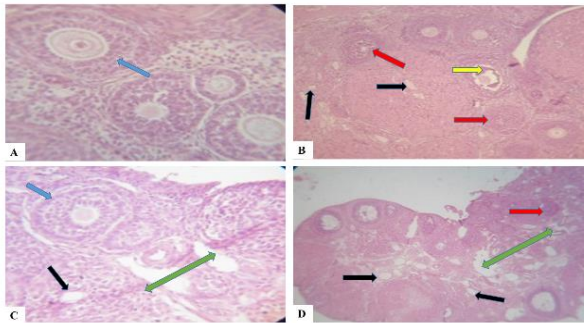


Figure 5. Photomicrograph of Ovarian sections of Wistar rats given sugar. A) Control (B) 6.25 mg/Kg sugar (C) 20 mg/Kg sugar (D) 64 mg/Kg sugar. The primary follicles appear normal (blue arrows). Atretic follicles (red arrows), distorted granulosa cells (yellow arrow), distorted stroma (green arrows) and vacuolations (black arrow) appear in the ovary. H&E x40 magnification.

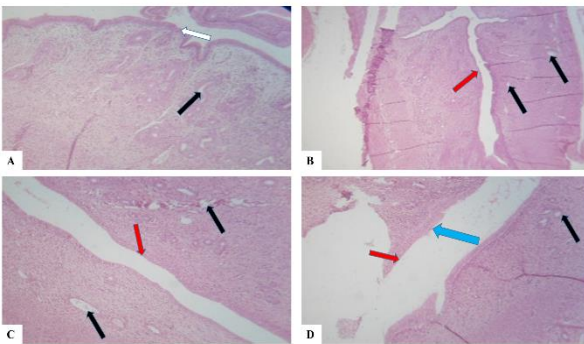


Figure 6. Photomicrograph of Uterine sections of Wistar rats given sugar. A) Control (B) 6.25 mg/Kg sugar (C) 20 mg/Kg sugar (D) 64 mg/Kg sugar. The lumen appears with a thick epithelial lining (white arrow), endometrial glands (black arrow) thin epithelial lining (red arrow) and widen uterine lumen (blue arrow). H&E x40 magnification.

Discussion

The fasting blood glucose level determined whether sugar intake may predispose the rats to hyperglycemia. The result of the study revealed that the lower doses of sugar significantly decreased the fasting blood glucose level, implying that this effect of sugar is probably dose-related. Also, it is plausible that the administration period influenced the result, as a longer period of the administration may show a significant difference (25, 26). Sugar treatment did not cause significant alterations in body weight gain of the animals. Though the higher doses of sugar both caused a significant reduction in the relative weights of the ovary and uterus in support of the findings of (27) who observed diminished ovary size in female drosophila under high-sucrose conditions.

The serum level of follicle-stimulating hormone increased significantly after treatment with the highest dose of sugar, contrasting the findings of (28) who reported a decrease in the follicle-stimulating hormone after treatment with high glucose due to decreased responsiveness of the pituitary gland to release gonadotropin-releasing hormone (GnRH). A malfunction of the ovary perhaps caused the increased follicle-stimulating hormone level. Failure of the ovary to secrete enough estrogen can adversely alter the correct feedback control of follicle-stimulating hormone production from the pituitary gland causing a rise in follicle-stimulating hormone level (29).

The vaginal smear of the sugar treated groups showed four phases of the estrous cycle with the right proportion of epithelial cells. The phases are proestrus (more of nucleated epithelial cells), estrus (majorly anucleated cornified cells), metestrus (presented with leukocytes, cornified and nucleated epithelial cells), and diestrus (mainly leukocytes cells) in line with the findings of (22). The estrous cycle was reported to be disrupted by genetic, environmental, age, and nutritional factors, which act at different levels of the hypothalamic-pituitary axis or at the organ level to inhibit ovulation (30). Though sugar intake did not significantly alter the length of the estrous cycle, it caused a significant decrease in the frequency of occurrence of the estrus phase and a concurrent increase in the frequency of occurrence of the metestrus and diestrus phases. This may result from a disruption in the function of the hypothalamic-pituitary axis, shown by the increased follicle-stimulating hormone level secreted from the anterior pituitary alongside the absence of its proper feedback mechanism (29) which perhaps altered estrogen secretion that in turn disrupted the oestrus cycle (28).

Cumulus granulose cells normally metabolize glucose into pyruvate for use by the oocyte and increase progesterone level (31), this may have led to the increased frequency of the metestrus phase. Furthermore, the prolonged metestrus phase can cause

pseudo-pregnancy (32) resulting from constant cervical stimulation during the collection of vaginal smear (29).

The sugar treated groups showed distorted granulosa cells, stroma cells with some vacuolations, and degeneration of ovarian follicles (atretic follicles), possibly due to the inability of the theca cells to secrete enough estradiol in response to the increased follicle-stimulating hormone level (33) as noted in the insignificant decrease in estradiol level of the sugar treated groups. Also, it is plausible that this can cause ovarian malfunction resulting in a disrupted estrous cycle (menstrual disorder), known to decrease the fertility rate in women. Usually, the ovary naturally stops functioning at menopause, but when it stops early it results in premature ovarian failure/insufficiency as seen in polycystic ovary syndrome (34). In this syndrome, the follicles mature to a certain stage, then stops growing and fail to release an egg. Studies have reported that follicular atresia limits the number of eggs supported for maturation and ovulation (35). A high-sucrose diet disrupts mature egg production, ovarian lipid, and sterol homeostasis in the female *Drosophila Melanogaster* model (36). Normally estradiol hormone thickens the uterine lining to prepare it for possible implantation of a fertilized egg (37), instead, the uterine sections of the sugar groups presented with thin uterine epithelial lining and wide lumen, which probably resulted from the lower level of estradiol observed. Also, the endometrium of the highest sugar-dosed group showed fewer uterine glands, which could cause a decrease in uterine secretions essential for reproduction thus, this might lead to difficulties in getting pregnant (38, 39). The abnormal shape of the uterine lumen may prevent the fetus from attaching to the endometrial wall, consequently leading to abortion or miscarriage.

Conclusion

Sugar intake increased the serum follicle-stimulating hormone level, disrupted the estrous cycle, altered the weights and cytoarchitecture of the ovary and uterus. Hence, sugar consumption has an adverse effect on the reproductive functions of female Wistar rats.

References

- World Health Organization. Sexual and reproductive health. 2013. Available from www.who.int/reproductivehealth/topics/infertility.html (Accessed October 3, 2013).
- Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Science*. 2015; 8:191-196.
- Cetin I, Berti C, Calabrese S. Role of micronutrients in the periconceptional period. *Human Reproduction Update* 2010; 16:80-95.
- Romero R, Romero G, Aortes I, et al. Risk factors associated to female infertility. *Ginecologia Obstetricia de Mexico* 2008; 76:717-721.
- Domar AD, Conboy L, Denardo-Roney, J, et al. Lifestyle behaviours in women undergoing in vitro fertilization: a prospective study. *Fertility and Sterility* 2012; 97:697-701.
- Pasquali R, Gambineri A. Metabolic effects of obesity on reproduction. *Reproductive Biomedicine Online*. 2006; 12:542-551.
- Chen JCP, Chou CC. *Cane Sugar Handbook: A Manual for Cane Sugar Manufacturer and Chemists*. John Wiley and Sons, Incorporated, New York, 1993;401-403.
- Shahbandeh M. Sugar cane production worldwide 1965-2018. The statista portal 2020.
- Taylor R. "2016 Outlook of the U.S. And World Sugar Markets, 2016-2025." North Dakota State University, Center for Agricultural Policy and Trade Studies 2016.
- Johnson RK, Appel LJ, Brands M, et al. American Heart Association Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism and the Council on Epidemiology and Prevention. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. *Circulation* 2009;15 (120):1011-1020.
- Westenhoefer J. Carbohydrates and cognitive performance. *Aktuelle Ernährungsmedizin*. 2006; 31:96-102.
- Nmadu J, Ojo MA, Ibrahim FD. Prospects of sugar production and imports meeting the sugar demand of Nigeria year 2020. *Russian Journal of Agriculture and socio-economic Science*. 2013; 14:15-25.
- Baghurst K, Baghurst P, Record S. Demographic and nutritional profiles of people consuming varying levels of added sugars. *Nutrition Research*. 1992; 12:1455-1465.
- Ogunwale E, Kunle-Alabi OT, Opeyemi OA et al. *Saccharum officinarum* molasses adversely alters reproductive functions in male wistar rats. *Toxicology Reports*. 2020; 7:345-352.
- Adekunbi DA, Ogunsola OA, Oyelowo OT, et al. Consumption of high sucrose and/or high salt diet alters sperm function in male Sprague-Dawley rats. *Egyptian Journal of Basic and Applied Science* 2016; 3:194-201
- Ruff JS, Suchy AK, Hugentobler SA, et al. Human-relevant levels of added sugar consumption increase female mortality and lower male fitness in mice. *Nature Communications*. 2013; 4:2245.
- Smith S, Pfeifer SM, Collins JA. Diagnosis and Management of Female Infertility. *Journal of America Medical Association* 2003; 290(13):1767-1770.
- Kelly D. Sugary drinks may slow sperm. *Live Science*. 2014.
- Rakesh K. The sweet poison also known as sugar and its effect on male fertility. 2014.
- World Health Organization. Fact sheet N°394 2015. <http://www.who.int/mediacentre/factsheets/fs394/en/>. Accessed October 10, 2020.
- Organisation for Economic Co-operation and Development Test Guideline 425. Acute Oral Toxicity Statistical Programme (AOT 425 Stat Pgm). Version: 1.0. 24: 2001;6775-6778.
- Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: Some helpful considerations. *Brazilian Journal of Biology*. 2002; 62:609- 614.
- Long JA, Herbert ME. *The oestrous cycle in the rat and its associated phenomena*. Berkeley, Calif: University of California Press, 1922.
- Mandl AM. The phases of the oestrous cycle in the adult White rat. *Journal of Experimental Biology*. 1951; 28:576-584.

25. Aeberli I, Hochuli M, Gerber PA, et al. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. *Diabetes Care* 2013; 36:150-156.
26. Heden TD, Liu Y, Park YM, et al. Moderate amounts of fructose or glucose-sweetened beverages do not differentially alter metabolic health in male and female adolescents. *American Journal of Clinical Nutrition*. 2014; 100:796–805.
27. Bastock R, Johnston D, Huynh JR, et al. *Drosophila* oogenesis. *Current Biology* 2008; 18: R1082-1087.
28. Christine C, Eric J, Lucie T, et al. Effects of high levels of glucose on the steroidogenesis and the expression of adiponectin receptors in rat ovarian cells. *Reproductive Biology and Endocrinology, BioMed Central*. 2008; 6(11):1-14.
29. Jeremy S. Oestrus Cycle learning module, 2018.
30. Astwood EB. Changes in weight and water content of uterus of the normal adult rat. *American Journal of physiology* 1939; 126:162-170.
31. Dalton CM, Szabadkai G, Carroll J. Measurement of ATP in single oocytes: impact of maturation and cumulus cells on levels and consumption. *Journal of Cell Physiology* 2014; 229:353-361.
32. Johnson MH. *Essential Reproduction*. 6th ed. New York, Y: Wiley-Blackwell. 2007; 316.
33. Findlay JK, Liew SH, Simpson ER, et al. Oestrogen signalling in the regulation of female reproductive functions. *Handbook of Experimental Pharmacology*. 2010; 198:29-35.
34. Alsadi B. Polycystic Ovarian Syndrome: Pathophysiology and Infertility. *World Journal of Laparoscopic Surgery*. 2014; 7:23-27.
35. Abreu MR Garcia P, Zaniboni-Filho, E. Histological characterization of oocyte developmental stages of *suruvi Steindachneridion scriptum* kept in captivity. *Acta Scientiarum Animal Sciences* 2015; 37:351-356.
36. Brookheart RT, Swearingen AR, Collins CA, et al. High-sucrose-induced maternal obesity disrupts ovarian function and decreases fertility in *Drosophila melanogaster*. *Biochimica et Biophysica Acta- Molecular Basis of Disease*. 2017; 1863:1255-1263.
37. Ozturk S, Demir R. Particular functions of estrogen and progesterone in establishment of uterine receptivity and embryo implantation. *Histology and Histopathology* 2010; 25:1215-28.
38. Gray CA, Bazer FW, Spencer TE. Effects of Neonatal Progesterin Exposure on Female Reproductive Tract Structure and Function in the Adult Ewe. *Biology of Reproduction*. 2001; 64:797–804.
39. Cooke PS, Ekman GC, Kaur J, et al. Brief exposure to progesterone during a critical neonatal window prevents uterine gland formation in mice. *Biology of Reproduction*. 2012; 86:63.