
**MATERNAL CONSUMPTION OF GLYCINE MAX AND SESAMUM INDICUM
INCREASES REPRODUCTIVE HORMONES IN FEMALE OFFSPRING OF
WISTAR RATS.**

Jesse-Abraham, Piriye Charit

Department of Science Laboratory technology,
School of Science and Technology,
Captain Elechi Amadi Polytechnic,
Rumuola, P.M. 5936 Port Harcourt
Email: junepipi@gmail.com.

&

Amah-Tariah, Fortune

Department of Human Physiology,
Faculty of Basic Medical Sciences,
University of Port Harcourt,
East West Road, P.M.B, 5323,
Choba Port Harcourt.
Email: fortune.amahtariah@uniport

Abstract

Soya Bean (Glycine max) and Sesame seed (Sesamum indicum) are bio-resources normally consumed freely, even during the critical window of pregnancy and lactation. The health benefits of Soya Bean and Sesame seed were investigated on the offsprings of female Wistar rats. Fifteen (15) Male and thirty (30) female Wistar rats were paired at a ratio of 1:2 to procreate 30 female pups. The adult rats were divided into 6 groups: Group 1 served as Control, Group 2 had Clomiphene, Groups 3 and 4 received 50mg and 100mg Soya Beans respectively, while group 5 and 6 received 50mg and 100mg Sesame Seeds respectively. After a 9week experimental period, the study animals were sacrificed and blood samples taken, tissues harvested under standard procedures and analyzed. The results showed that 50mg Sesame seed group showed significant increase in LH (1.58 ± 0.40)mlu/ml, when compared to the control (0.45 ± 0.08) mlu/ml significance was considered at $p < 0.001$ and Progesterone level for 100mg Sesame seed (32.46 ± 2.00)ng/ml, when compared to the control, (14.98 ± 2.27)ng/ml, significance was considered at $p < 0.001$. The increase in LH levels signifies the onset of puberty while increased levels of progesterone can lead to anxiety, disruption in the onset of puberty and the menstrual cycle. The study therefore, concludes that graded doses of Sesamum indicum is capable of increasing the levels of LH and Progesterone and causing changes to offsprings when consumed maternally.

Introduction

Bio-resources are all living-based matter that results directly or indirectly from photosynthesis that is any reserve obtained from biological origin or nature. They are materials generated from nature and are sustainably renewable and biodegradable. Bio-resources are numerous and include agricultural crops, forestry products, and biologically-derived waste, marine resources like fishes, weeds, grasses etc. Bio-resources are valuable economic raw materials for food and industries. Soya Bean and Sesame seeds are bio-resources normally consumed freely, even during the critical window of pregnancy and lactation. Nigeria is rich in several categories of bio-resources hence bio-resources should be employed to solve the food, environmental and numerous health problems of this nation. This paper highlights the use of Soya bean and Sesame seed as an alternative to routine drugs to improve fertility in female offsprings of Wistar rats, (Avinash, *et. al.*, 2020).

Soya bean, Soy or Soybean (*Glycine max*) is a leguminous plant generally cultivated for its numerous uses such as soya milk, fermented foods such as soy sauce, fermented bean paste, soy oil, animal feeds and it is the main protein source in many packed meals such as textured vegetable protein (TVP) used in numerous meat and dairy alternatives for vegetarians. Industrially, Soy bean is used for oils, solvents, crayons, cosmetics, resins, plastics, inks, soap, clothing, biodiesel and in the production of Vodka. Soya bean can trigger reactions in some persons especially in children, (Riaz, 2006). Sesame (*Sesamum indicum*) is a flowering plant and one of the oldest edible tiny oilseed plants in the world with several health benefits such as improving digestion, skin and hair health, reducing hypertension and inflammation, cancer prevention, improving male fertility, prevention of bone diseases, controlling diabetes and improving oral health. Sesame seed is cultivated in tropical areas round the world; top producers are Tanzania, Myanmar, India and Sudan. Sesame seed is an oil seed crop famous for tolerating drought conditions or other vagaries of weather that other plants do not thrive in (Ogasawara *et al.*, 1988; Raghav *et al.*, 1990). Sesame seed oil contains a rich source of most inorganic nutrients with remarkable levels of essential minerals like zinc, calcium and phosphorus. These minerals are essential for creating new bone and repairing injured or weakened bones due to diseases like osteoporosis (Johnson *et al.*, 1979). Osteoporosis is characterized by reduced bone density an ailment that affects bone health as one age and increases the risk of fracture. Oestrogen deficiency in menopausal women can cause this condition because oestrogen help maintain normal bone density and this can be remedied by taking hormone replacement therapy to help promote bone strength but this comes with some adverse side effects.

Phyto-oestrogens have been purported to be a natural substitute although research from animal studies have shown to have bone sparing effect on rats but potency depends on dose, route and duration of administration, (Dodge *et al.*, 1996; Draper *et al.*, 1997; Anderson *et al.*, 1998; Cassidy *et al.*, 2006). An Iranian study used 50 people with knee osteoarthritis were divided into two: control and sesame group. The sesame group was administered 40grams/day of powdered sesame a day to their usual treatment (acetaminophen and glucosamine) for two months and they reported greater pain relief than those just on their usual treatment (Eftekhar *et al.*, 2013). Consuming moderate amounts of sesame seeds daily can prevent the development of bone health deficiencies. The effect of soya bean for the potential suppression of the adult hypothalamic-pituitary-gonadal (HPG) axis has been revealed by studies carried out on both humans and animals. Several studies have reported that phyto-oestrogens mimic the activity of oestrogen in ovariectomized rodents (Degen *et al.*, 2002; Schmidt *et al.*, 2006) and in humans' intake of isoflavones-rich soy foods suppress circulating oestrogen and progesterone concentrations and can assuage the pre-ovulatory surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Santell *et al.*, 1997;

Duncan *et al.*, 1999; Wu *et al.*, 2000). However, some findings have not found any impact of isoflavones on female hormone levels.

A recent study that administered 100mg of isoflavones per day to 34 women for a year, found no changes in the luteal phase levels of oestradiol, oestrone, progesterone, SHBG, FSH or LH at months 1, 3, 6 or 12 (Maskarinec *et al.*, 2002). Soy cookies with about 52mg of isoflavones or isoflavone-free cookies were given to 14 women for 5 days, produced no alteration of circulating gonadal hormone levels. It is worthy of note that ethnicity maybe an undertone factor, when evaluating the influence of soy isoflavones. A study found suppressed luteal oestradiol levels following soy intake only in women of Asian descent (Wu *et al.*, 2000).

Another study done in pre-menopausal females reported that, isoflavone consumption suppresses LH and FSH levels and increases cycle duration (Hooper *et al.*, 2009). This contradicts the clinical occurrence reported from Suny Downstate Medical Center (Chandrareddy *et al.*, 2008) that for females planning to get pregnant or facing menstrual cycle irregularities, foods containing soya bean should be avoided or used with caution. A different study investigated the effect of *Puereria mirifica* containing phytoestrogens on reproductive systems of both sexes of gonadectomized rats treated orally with 0, 10, 100 and 1000mg/kg Body Weight per day. The study concluded that a high dose (1000m/kg BW) of *P. mirifica* can cause abrupt increase in serum levels of FSH at day 1 and gradual increase of LH within an 8-day period. The study reported that *P. mirifica* can sway reproductive functions in both sexes of rats, but the response in female rats was greater than in male rats and the recovery was slower (Schinda *et al.*, 2004).

Research design

The work was carried out in the animal house of the faculty of Basic Medical Sciences, University of Port Harcourt.

Study animals

Male and female Wistar rats with weights between (130g-200g) were procured from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were kept in an environment of normal room temperature and fed with standard feed and water ad libitum for 14 days to allow for acclimatization before the experimental process began. After acclimatization the animals were weighed weekly, grouped and paired for mating during the oestrous period (heat) of females so pregnancy can be achieved. The mature female animals were divided into 6 groups: a control group, the clomiphene group, the low dose Soya bean group, the High dose Soya bean group, low dose Sesame seed groups and High dose Sesame seed. Two females were paired with a male rat in a cage and allowed to mate. On the commencement of pregnancy, validated by the presence of sperm in the vaginal fluid and the development of the vaginal plug, the males were removed from the cage and the Wistar rats in each group were fed with normal animal feed and water, group 2 to 5 were force-fed with their designated substrate according to the technique described by M. E Fowler (1981) with the slight modification of using a syringe instead of a speculum to intubate the test substance daily until the animals gave birth. Group 1 control were fed normal feed and water ad libitum, Group 2- Clomiphene group were force fed clomiphene citrate tablet dissolved in water for three days after which normal feed and water were administered daily, Group 3-Low dose Soya bean group: were force fed 50mg/kg weight soya beans powder, dissolved in water daily, Group 4- high dose Soya bean were force fed 100mg/kg body weight soya beans powder, dissolved in water, Group 5- Low dose Sesame seed group were force fed roasted ground sesame seeds, 50mg/kg weight dissolved in water and Group 6-the high dose Sesame seed group, were force fed roasted ground sesame seeds, 100mg/kg weight dissolved in water

Preparation of soya bean and Sesame seed

Soya bean and Sesame seed were bought from the Mile 1 market, Diobu Port Harcourt, animal feed was also purchased from Choba market and fed to the animals. The Soya bean was washed and dried by roasting it dry. The outer coat was removed and the Soya bean was ground to fine powder. The sesame seed was also washed roasted dry and milled to powder using the open pan technique described by Makinde *et. al.*(2016).

Sample collection and hormonal assay

Five weeks after the birth of the offsprings, blood samples were randomly taken from 5 female offspring from each group for serum hormonal assay (Oestrogen, Luteinizing Hormone, Follicle Stimulating Hormone and progesterone). The procedure involved anesthetizing the rats in a desiccator using cotton wool infused with chloroform. The animals were laid in a supine position and their limbs were pinned. Blood samples were collected by venipuncture, pelvic incisions were also made to expose the ovaries and uteri. A sharp scalpel was used to excise the organs (ovary and uterus) for histological profiling to ascertain if there is any evidence of hormonal disruptions during development. This is based on enzyme linked immunosorbent assays (ELISAs) principle. ELISA can be used to measure almost any protein, plus hormones. This test uses the specificity of antibodies sensitivity to simple enzymes. It is performed on plastic plates having 96 wells each. Every well is coated with an antibody (AB₁) that is specific for the hormone to be assayed. Samples or standards are added to each well, then a second antibody AB₂ that is also specific for the hormone but binds to a different site of the hormone molecule. A third antibody AB₃ is added and it recognizes AB₂, and is coupled to an enzyme that converts a suitable substrate to a product that can be easily detected by colorimetric or fluorescent optical methods. Small amounts of hormone molecules can be detected because each molecule of enzyme catalyzes the formation of many thousands of product molecules. ELISA methods in contrast to radioimmunoassay methods employ excess antibodies such that all hormone complexes are captured in antibody-hormone complexes. Consequently, the amount of hormone present in the sample or standard is proportional to the amount of product formed (Guyton *et. al.*, 2016)

Histological profiling.

Carleton's Method, (1967), was implemented in this work. The tissues (ovary, uterus and testes) were harvested after the animals were sacrificed and put in 10% formal saline until when they were ready to be processed. Dehydrated in graded concentrations of ethanol was done, 50%, 70%, 90%, 95% and lastly 100% for two hours. The tissues were passed in absolute alcohol twice. Xylene was used to clear by transferring them into equal volumes of 100% alcohol and xylene for one hour to avoid tissue damage. The tissues were then passed through changes of xylene for one hour each. The tissues were then embedded in two changes of paraffin wax at 55°C for two hours each. L-shaped metal blocks were filled with paraffin wax and the tissues were placed in it immediately with forceps, with the surface to be cut facing downwards. The paraffin was allowed to cool and formed a solid paraffin which was immersed in water. The blocks were then sectioned thinly using rotary microtome. The sections were put onto a slide that was floated in 20% alcohol and then in warm water bath. The sections were mounted on albumenized slides and dried in oven at 37°C overnight. Xylene was used twice to dewax and then dehydrated in descending grades of alcohol from absolute alcohol to 70% alcohol for one minute in each grade. The sections were then washed and stained with heamatoxylin for 20 minutes and washed again with water, 1% acid and alcohol was used to differentiate the tissue for five seconds and washed with water thereafter. The tissues were blued in ammonical water for two minutes, washed with water and concentrated with

1% aqueous eosin for minutes, rinsed with water and dehydrated again in ascending grades of alcohol starting from 70%, 90%, and then 100% for one minute in each grade.

The tissues were then cleaned in xylene covered with coverslip and finally mounted in DPX (distrene, triceresyl phospatexylene) and examined under the microscope after air drying them.

Data Analysis

Data was analyzed using GraphPad prism 6.0 statistical software. Data was presented as Mean \pm SEM. Significant differences was considered using one-way analysis of variance (ANOVA) followed by Tukey's post- hot test. Significance was considered at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

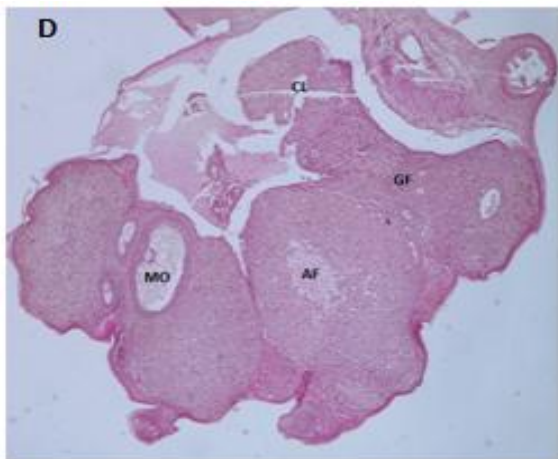
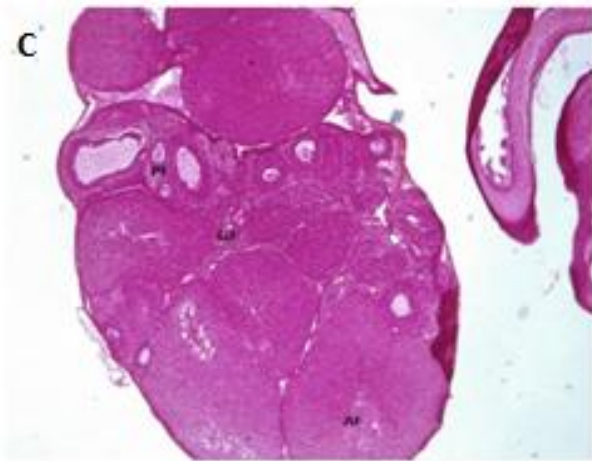
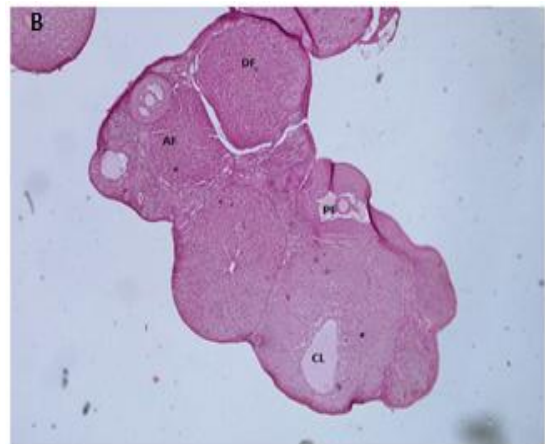
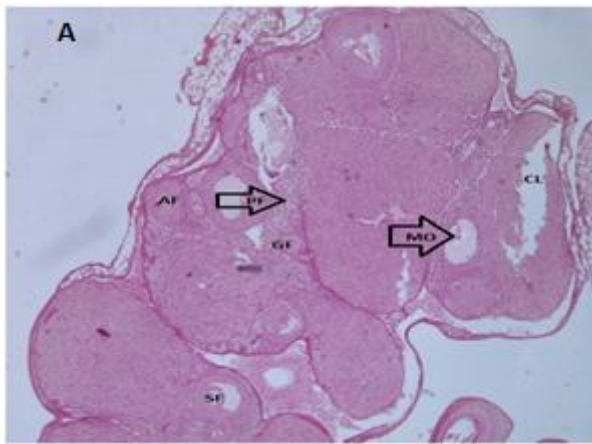
Results

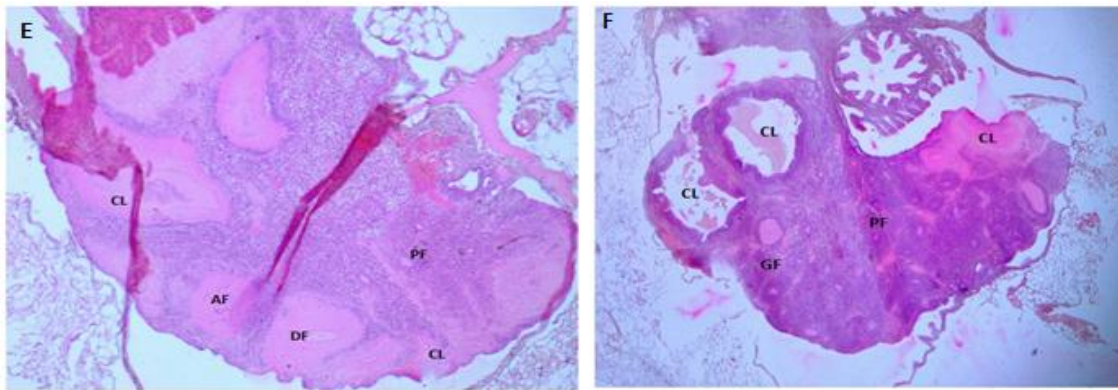
Table 1: Values of reproductive Hormones in female study offsprings.

Group	n	FSH (mlu/ml)	LH (mlu/ml)	E ₂ (ng/ml)	Prog (ng/ml)
1 Control	5	0.24 \pm 0.04	0.45 \pm 0.08	71.40 \pm 2.70	14.98 \pm 2.29
2 Clomiphene (mg)	5	0.23 \pm 0.03	0.88 \pm 0.11	83.00 \pm 3.81	12.37 \pm 2.78
3 50mg SB	5	0.25 \pm 0.06	1.02 \pm 0.09	72.40 \pm 4.59	14.38 \pm 1.40
4 100mg SB	5	0.29 \pm 0.04	0.64 \pm 0.10	86.20 \pm 7.69	23.96 \pm 3.44
5 50mg SS	5	0.21 \pm 0.01	1.58 \pm 0.40***	82.00 \pm 1.79	15.68 \pm 2.17
6 100mg SS	5	0.26 \pm 0.02	0.86 \pm 0.07	67.60 \pm 2.50	32.46 \pm 2.00***

Values are expressed as Mean \pm SEM, *** $p < 0.001$ when compared to control. FSH: Follicle stimulating hormone; LH: Luteinizing Hormone; E₂: Oestradiol; Prog: Progesterone; SB: Soya Bean; SS: Sesame Seed. The results of the administration of soya bean and Sesame seeds on the reproductive hormones of female offsprings of Wistar rats that were administered the test substances are presented in Table 1. It was observed that there was significant increase in LH for the 50mg Sesame Seed group (1.58 \pm 0.40) mlu/ml when compared to the control (0.45 \pm 0.08) mlu/ml, the level of significance was considered at $p < 0.001$. Progesterone levels also increased significantly in the 100mg Sesame Seed group (32.46 \pm 2.00) ng/ml when compared to the control (14.98 \pm 2.27) ng/ml, ($p < 0.001$). A variety of infertility problems may arise if the right amounts of LH are not produced at the right time; problems ranging from menstrual irregularities, premature or delayed puberty in children (medlineplus.gov, 2020).

A statistically significant increase in mean LH level was observed in the 50mg Sesame Seed (low dose) group when compared to the control. A study affirms our findings and reported an increase in reproductive hormones of rats treated with *Puereria mirifica* containing phytoestrogens (Schinda M. *et. al.*, 2004). A significant increase of progesterone was recorded in the 100mg Sesame Seed (high dose) group. Raised progesterone levels are linked with symptoms of premenstrual syndrome (PMS), such as distending stomach, breast tenderness and even mood swings (medlineplus.gov, 2020). This raised level may cause disruption in the onset of puberty and the menstrual cycle (that is ovulation may fail to occur). Raised levels of progesterone could also be associated with anxiety, and/or weight fluctuations. One study by Dagmar *et. al.* (2009) negates this result obtained in; human term trophoblast cells which reduced when treated with genistein and daidzein, however no other literature affirms or negates the results of this present study





Photomicrograph sections of normal ovaries Mag.X100, H & E stain. Plate A: control (Group1) .Section has ovarian cortex with primordial follicles (PF), growing follicles (GF) and mature ovum (MO), atretic follicles (AF), secondary follicles (SF) and Corpus Luteum at different stages of developments.

Plate B: Clomiphene group: Normal ovary section exhibiting more atretic follicles (AF), corpus luteum (CF), Primary Follicles (PF), degenerated follicles (DF) and corpus luteum (CL). Plate C: 50mgSB (Group 3): Normal ovary with more atretic follicles (AF) with few mature ova. There are also few primordial follicles (PF) and germinal follicles (GF).

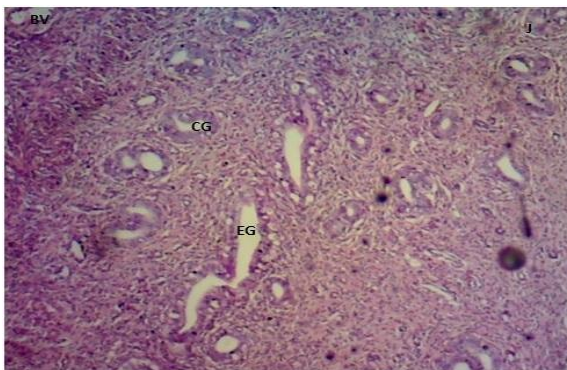
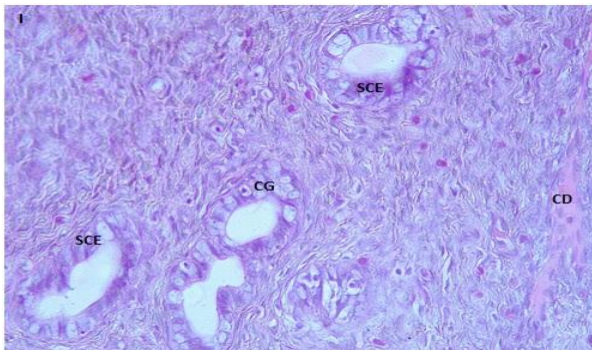
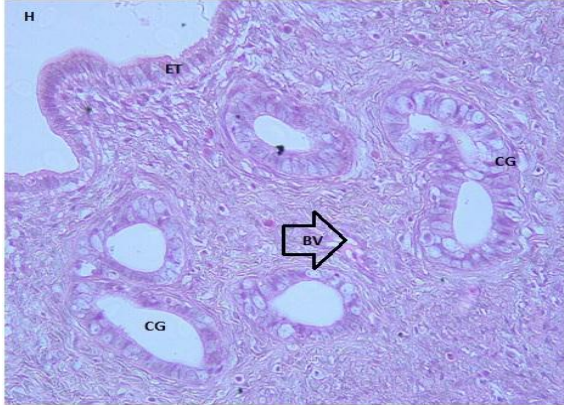
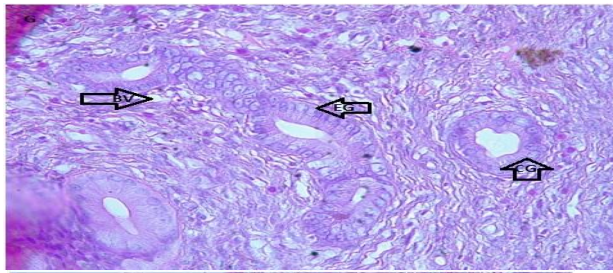
Plate D: 100mgSB (Group 4) -matured ova (MO), growing follicles (GF) and primordial follicles (PF), atretic follicles (AF) with more corpus luteum (CL).

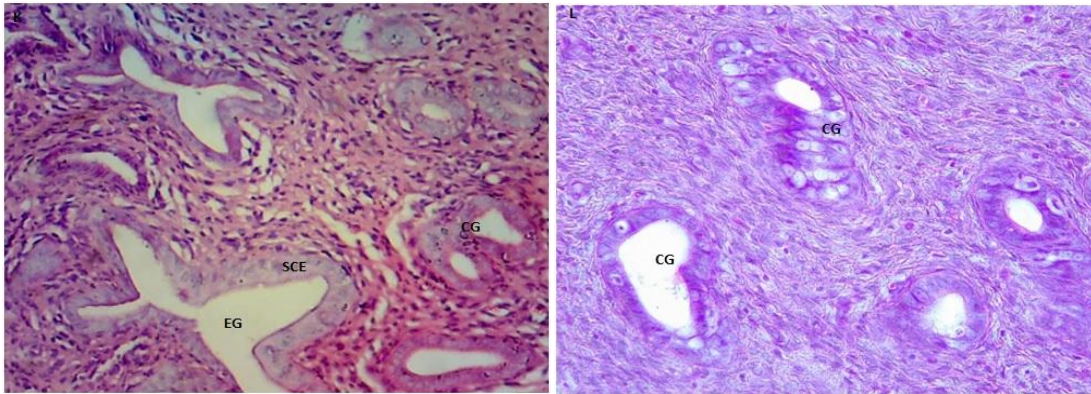
Plate E: 50mgSS (Group 5): Normal section showed corpus luteum with few primordial follicles (PF), germinal follicles (GF), atretic follicles, degenerating follicles and corpus luteum (CL).

PlateF: 100mgSS (Group 6): Normal ovary with corpus luteum(CL), primordial follicles (PF) and germinal follicles (GF). The primary follicles, secondary follicle and mature ova are degenerated.

G

H





Photomicrograph sections of uterus, Mag.X400, H & E stain. Plate G: Control (Group 1) Histologically normal uteri with the endometrium in proliferative phase, endometrial glands lined by simple epithelial cells, narrow and do not contain mucus secretions, with coiled gland (CG) and enlarged gland (EG), numerous blood vessels (BV) are present. Plate H (Group 2): Normal uterine tissues with the endometrium in the proliferative phase. Endometrial glands lined by simple columnar epithelial cells with coiled glands (CG), endometrial tissues with blood vessels (BV) and normal glandular epithelial tissues (ET). Plate I (Group 3) Uterine endometrial tissues with glandular epithelium-simple columnar epithelium (SCE).Some tissue degeneration and distortions (CD), are present. There is loss of glandular epithelial tissues.

Plate J (Group 4): Uterine tissue, are in the secretory phase. Epithelial lining is present in the coiled gland (CG) Uterine gland crypt present in the enlarged glands (EG) and numerous blood vessels. PlateK (Group 5): Uterine endometrial tissues in proliferative phase with no glandular secretions in the coiled glands .Enlarged gland (EG) present with poorly defined simple columnar epithelium (SCE). There are also few blood vessels. Plate L (Group 6): Section of Uterine tissue, with the endometrium in the proliferative phase. Coiled glands (CG) present in the endometrium. Few blood vessels present.

The histological profiling of the ovary appeared to be in the process of follicular atresia (degeneration) which may occur at any stage in the development of the ovum. By the sixth month of development, the foetal ovary contains several million primordial follicles, but by the time of birth less than half a million remain. Atresia continues until puberty and thereafter through the reproductive years. With each ovarian cycle approximately 20 follicles begin to mature; usually all but one becomes atretic at some stage before complete maturity. The histological profile of all the groups show that maturation of one follicle has not begun hence sexual maturity has not been attained; there was no evidence of Female reproductive tract abnormalities in all the groups. The uterus of all groups appeared normal in different stages of the cycle with the presence or absence of secretory gland cells.

Conclusion

Soya bean and Sesame seeds fed to pregnant Wistar rats could increase Luteinizing hormone and progesterone levels in female offspring and may be the cause of premature or delayed onset of puberty and menstrual irregularities in female offspring.

References

- Adewale, H. B., Jefferson, W. N., Newbold, R. R. & Patisaul, H. B., (2009). Neonatal bisphenol-A exposure alters rat reproductive development and ovarian morphology without impairing activation of gonadotropin releasing hormone neurons. *Biology Reproduction*, 81(4), 690-699.
- Anderson, J. J., Ambrose, W.W. & Garner S. C., (1998). Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. *Proceedings of the Society for Experimental Biology and Medicine* 217:345–350.
- Bateman, H. L. & Patisaul H. B., (2008). Disrupted female reproductive physiology following neonatal exposure to phytoestrogens or estrogen specific ligands is associated with decreased GnRH activation and kisspeptin fiber density in the hypothalamus. *Neurotoxicology*. 29:988–997.
- Balk, E, Chung, M. Chew, P. I. S, Raman, G, Kupelnick, B, Tatsioni A, Sun Y, Devine, D. & Lau, J. (2005). Effects of soy on health outcomes. *Evidence Report/Technology Assessment (Summ)* 1–8.
- Cassidy, A, Albertazzi, P, Lise, Nielsen I., Hall, W, Williamson, G., Tetens, I., Atkins, S., Cross H., Manios, Y., Wolk, A., Steiner, C. & Branca, F. (2006). Critical review of health effects of soyabean phyto-oestrogens in post-menopausal women. *Proceedings of Nutrition Society*. 65, 76–92.
- Chandrareddy, A., Muneyyirci-Delale, O., McFarlane S.I. & Murad O. M. (2008). Adverse effects of phytoestrogens on reproductive health: a report of three cases. *Complement Therapies in Clinical Practice* 14, 132–135.
- Cooke, G. M. (2006). A review of the animal models used to investigate the health benefits of soy isoflavones. *J. AOAC International* 89, 1215–1227.
- Crain, D. A, Janssen, S. J., Edwards, T. M., Heindel, J., H. S. M., Hunt. P., Iguchi, T., Juul, A., McLachlan, J. A., Schwartz, J., Skakkebaek, N., Soto, A. M, Swan, S, Walker., C, Woodruff TK, Woodruff, T. J, Giudice L. C, & Guillette L. J., Jr., (2008). Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. *Fertility Sterility*. 90, 911–940.
- Dagmar, U. R., Loanis, M., Bettina, T., Christoph, S., Volker, B., Klaus, F., & Udo, J., (2009). Effects of phytoestrogens genistein and daidzen on progesterone and estrogen (estradiol) production of human term trophoblast cells in vitro. *Gynaecological Endocrinology*, 25(1), 32-38. doi.org/10.1080/09513590802485020.
- Degen, G. H., Janning, P., Diel, P. & Bolt, H. M., (2002). Estrogenic isoflavones in rodent diets. *Toxicology Letter*, 128, 145–157.
- Dodge, J. A., Glasebrook A. L., Magee D. E., Phillips D.L., Sato M., Short L. L., Bryant H. U., (1996) Environmental estrogens: effects on cholesterol lowering and bone in the ovariectomized rat. *Journal of Steroid Biochemistry and Molecular Biology*, 59, 155–161.
- Draper, C. R., Edel, M. J., Dick, I. M., Randall, A. G., Martin, G. G. & Prince, R.L., (1997) Phytoestrogens reduce bone loss and bone resorption in oophorectomized rats. *Journal of Nutrition*, 127, 1795–1799.
- Duncan, A. M., Merz, B. E., Xu, X., Nagel, T. C., Phipps, W. R. & Kurzer, M. S., (1999). Soy isoflavones exert modest hormonal effects in premenopausal women. *Journal Clinical Endocrinology and Metabolism*, 84, 192–197.
- Eftekhari, S. B., Khadem, H. M, Alipoor, B., Malek, M. A., Asghar, J. M. & Moghaddam A., (2013). Effects of sesame seed supplementation on clinical signs and symptoms in patients with knee osteoarthritis. *International Journal of Rheumatic Diseases*, 16(5), 578-82. doi 10:111/1756-185x.12133.

- Faber, K. A., & Hughes, C. L. Jr., (1993). Dose-response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats. *Reproductive Toxicology*, 7, 35–39.
- Fowler, M. E., (1981). Force feeding techniques in wild animals. *The Journal of Zoo Animal Medicine*, 12 (1), 3-10.
- Gangur, V., Kelly C., & Navuluri, L., (2005). Sesame allergy: a growing food allergy of global proportions? *Ann Allergy Asthma Immunology*, 95(1), 4-11.
- Guyton, A. C. & Hall J. E., (2006). *Textbook of Medical Physiology*. (11th edition). Philadelphia. Elsevier Saunders. ISBN 0-8089-2317-X.
- Hooper, L., Ryder, J. J., Kurzer, M. S., Lampe, J. W., Messina, M. J., Phipps, W. R. & Cassidy A., (2009). Effects of soy protein and isoflavones on circulating hormone concentrations in pre- and post-menopausal women: a systematic review and meta-analysis. *Human Reproduction*, 15, 423–440.
- Jacobs, A., Wegewitz, U, Sommerfeld, C., Grossklaus, R. & Lampen A., (2009) Efficacy of isoflavones in relieving vasomotor menopausal symptoms - a systematic review. *Molecular Nutrition Food Research*, 53, 1084–1097.
- Jefferson, W. N., Couse, J. F., Padilla-Banks, E., Korach, K. S. & Newbold, R. R., (2002). Neonatal exposure to genistein induces estrogen receptor (ER) alpha expression and multiocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biology Reproduction*, 67, 1285–1296.
- Jefferson, W. N., Padilla-Banks, E., Goulding, E. H., Lao, S. P., Newbold, R. R. & Williams, C. J., (2009). Neonatal exposure to genistein disrupts ability of female mouse reproductive tract to support preimplantation embryo development and implantation. *Biology Reproduction*, 80, 425–431.
- Joensen, U. N., Jorgensen, N., Rajpert-De, M. E. & Skakkebaek, N. E. (2008). Testicular dysgenesis syndrome and Leydig cell function. *Basic Clinical Pharmacology and Toxicology*, 102, 155–161.
- Kim, J. (2008). Protective effects of Asian dietary items on cancers - soy and ginseng. *Asian Pacific Journal for Cancer Preview*, 9, 543–548.
- Kouki, T., Kishitake, M., Okamoto, M., Oosuka I., Takebe M. & Yamanouchi K., (2003). Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. *Hormonal Behaviour*, 44, 140–145.
- McLachlan, J. A., Newbold, R. R. & Bullock, B. C., (1980) Long-term effects on the female mouse genital tract associated with prenatal exposure to diethylstilbestrol. *Cancer Research*. 1980, 40:3988–3999.
- Makinde, F. M. (2016). Influence of roasting techniques on chemical composition and physico-chemical properties of sesame (*sesamum indicum*) seed flour and oil. *Applied Tropical Agriculture* 21(2), 25-31.
- Maskarinec, G., Williams, A. E., Inouye, J. S., Stanczyk, F. Z. & Franke A. A. (2002) A randomized isoflavone intervention among premenopausal women. *Cancer Epidemiology Biomarkers Prevention*, 11, 195–201.
- Messina, M., Gardner, C. & Barnes S. (2002). Gaining insight into the health effects of soy but a long way still to go: commentary on the fourth international symposium on the role of soy in preventing and treating chronic disease. *Journal of Nutrition*, 132, 547S–551S.
- Ogasawara, T., Chiba, K. & Tada, M. (Y. P.S. Bajaj ed.) (1988). *Medicinal and Aromatic Plants*, 10. Springer, ISBN 978-3-540-62727-2.
- Patisaul Heather B. & Jefferson, W. (2010). “The pros and cons of phytoestrogens”. *Frontiers in Neuroendocrinology*, 31(4), 400-19.

- Raghav, R., David C., Juan R., & Craig C., (1997). Sesame: New approaches for crop improvement and advances in new crops. 225-228. Timber press, Portland, OR.
- Riaz, Mian N., (2006). *Soy applications in food*. Boca Raton, FL: CRC Press. ISBN 978-0-8493-2981-4.
- Sacks, F. M, Lichtenstein, A., Van Horn. L., Harris, W, Kris-Etherton, P. & Winston, M. (2006). Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the *Nutrition Committee*. *Circulation*. 113:1034–1044.
- Santell, R. C., Chang, Y. C., Nair, M. G., Helferich, W.G., (1997). Dietary genistein exerts estrogenic effects upon the uterus, mammary gland and the hypothalamic/pituitary axis in rats. *Journal of Nutrition*. 127:263–269.
- Schmidt, S., Degen, G. H., Seibel, J., Hertramp, T., Vollmer G. & Diel P., (2006). Hormonal activity of combinations of genistein, Bisphenol A and 17beta-estradiol in the female Wistar rat. *Archives of Toxicology* 80:839–845.