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Comparative Effect of Oral Administration of Some Dietary Lipids on Fertility Hormones of Female Wistar Albino Rats

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Abstract: The effect of oral administration of soybean oil, olive oil and margarine on hormonal responses was examined on Wistar albino rats. Female rats were administered the three different dietary lipids orally for 21 days, while the control group received only water. Reproductive hormones: testosterone, progesterone, estradiol, follicle stimulating hormone, luteinizing hormone and prolactin levels were assayed by enzyme linked immunosorbent assay method using assay kits. Data were analyzed by ANOVA test and $p<0.05$ was considered significant. From the results, serum testosterone level showed a significant ($p<0.05$) decrease in the group given with soybean oil (41.31±3.18 ng/ml) accompanied by a non-significant ($p>0.05$) decrease in the groups treated with olive oil (44.78±2.18 ng/ml) and margarine respectively in relative to control group (46.00±2.39 ng/ml) There was a non-significant ($p<0.05$) decrease in serum progesterone level in the groups treated with soybean oil (6.68±0.42 ng/ml) and olive oil (6.72±0.45 ng/ml) accompanied by a non-significant increase in margarine (7.75±1.36 ng/ml) treated group respectively in comparison with the control group (6.47±0.42 ng/ml). Furthermore, serum estradiol level was non-significantly ($p>0.05$) increased in the groups administered soybean oil (1.64±0.28 pg/ml) and margarine (1.65±0.10 pg/ml) accompanied by a non-significant ($p>0.05$) difference in the olive oil (1.58±0.12 pg/ml) treated group relative to the control (1.54±0.32 pg/ml) group. Also, serum luteinizing hormone level was significantly ($p<0.05$) increased in the group given with olive oil (20.57±15.00 mIU/mL) followed by a non-significant ($p>0.05$) difference in the groups treated with margarine (15.71±6.70 mIU/mL) and soybean oil (15.46±1.76 mIU/mL) relative to the control (15.18±3.58 mIU/mL) group. Similarly, serum follicle stimulating hormone level was significantly ($p<0.05$) increased in the olive oil (17.16±10.81 mIU/mL) and soybean oil (17.08±7.62 mIU/mL) treated groups accompanied by a non-significant ($p>0.05$) increase in the margarine (14.38±7.26 mIU/mL) treated group relative to the control (12.41±4.44 mIU/mL) group. There was a significant ($p<0.05$) increase in serum prolactin level in the groups administered olive oil (12.19±9.70 ng/ml) and margarine (12.19±6.84 ng/ml) accompanied by a non-significant ($p>0.05$) increase in soy oil (7.78±3.41 ng/ml) treated group relative to the control (5.71±2.40 ng/ml) group. Based on the results, it is indicative that the three different dietary lipids especially olive oil and soybean oil have strong capability of enhancing hormonal functions by stimulating hypothalamus-pituitary ovarian axis and subsequently the fertility of females. Hence, consumption of these lipids could reduce risk of infertility in females.

Key words: Fertility Hormones · Luteinizing Hormones · Prolactin · Dietary Oil · Reproductive Hormones

INTRODUCTION

Fertility hormones regulate the reproductive cycle and are used to test for various associated conditions including infertility and impotence in men and women, early or delayed puberty as well as non reproductive disorder. The most common causes of female infertility are hormones. These are commonly associated with ovulation, ovarian syndrome, premature ovarian failure, damage to the Fallopian tube or uterus, or problem with cervix. Endocrine disorders results from excessive production of hormones, or insufficient production of one or more hormones or the lack of the tissues responses to normal circulating hormones [1]. The female reproductive

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cycles function primarily by the interplay between the luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol, prolactin and testosterone. Also, the integrity of the female reproductive organs can be assayed by the serum level of these hormones [2]. Lipids are essential component of the diet, as they are among the major sources of energy second to carbohydrate. Lipids are required for the absorption and transport of lipid soluble vitamins through the blood stream [3,4].

Nutrition and reproduction have always been linked in that the reproductive success of an animal depends on its nutritional status. Through the years, this link has been explored in research, often by altering diets in various ways and observing the resulting changes in reproductive parameters. One of the most significant dietary changes that can be made to influence the reproductive system is the addition of fat to the diet. Several studies on reproductive activity of fat-supplemented rats demonstrated an increase in diameter and number of follicles present on the ovary, as well as a shorter period to the first postpartum ovulation [5 ,6]. Originally, it was believed that the improvement in reproduction due to the addition of fat was solely a result of an increase in energy availability to the animal. However, it soon became apparent that individual fatty acids themselves can play a role in influencing reproductive parameters [7].

Different types of fats have been utilized in an attempt to improve reproductive function in animals. Fatty acids derived from plants and oil seeds have exerted a major impact on reproductive performance; some of the most common sources include sunflower, linseed, cottonseed, rape-seed and soya bean. Early studies on the effect of fat in the ration on reproductive performance were carried out by Burr and Bur [3] and Hirschfield [8], who observed that fat deficiency in the ration of growing rats induced alterations in ovulation rate and on the onset of oestrus, while lipid supplementation reestablished reproductive performance of the females, coining the concept of essential fatty acids. In later studies, research was aimed at evaluating the effect of fat supplementation in different animal species both ruminant and non-ruminant on reproductive aspects such as the establishment of puberty, recognition of pregnancy [9-11], by means of the suppression of luteolytic signals, restart of ovarian activity after parturition, follicle development, quality of oocytes and of the embryo, modification in the mechanism of synthesis and secretion of hormones involved in reproductive processes [12]; Staples *et al.* [13] and Litwack and Schmidt [14] and on production aspects such as quality of milk or meat. A recent review on the effects of dietary fat on fertility of dairy cows showed that 11 of 20 articles reported improvement [15,16]. Authors suggest that this effect was probably not a result of improvement of the energy status of the cows but that increased fertility could be due to effects of dietary fatty acids on the pituitary, ovaries and uterus. However, a hormonal effect of dietary lipid does not appear to have received an indepth studies. There is therefore the need to understanding the effect of some dietary lipids on serum fertility hormones in rats.

MATERIALS AND METHODS

Procurement of Dietary Lipids: Soybean oil (100% purity), olive oil (virgin brand) and margarine (blue band) were bought from the Umuahia market, Abia State. These were the three sources of dietary lipids (polyunsatuared fatty acid, monounsaturated fatty acid and saturated fatty acids respectively) used for this study.

The Experimental Animals: Sixteen female Wistar albino rats weighing 120 – 140g (12 – 13 weeks old) were purchased from the laboratory animal center of department of Zoology, University of Nigeria, Nsukka, Enugu State. They were properly housed in clean cages. The rats were fed on standard diet (vital grower mash); water was given *ad libitum* and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25 – 27°C under day/night 12-12h photoperiodicity. The animals were acclimatized for two weeks before used for the experiments.

Experimental Design: The animals were grouped into four groups which comprises of 4 animals each (A, B, C and D). Group A (control group) received standard feed and normal tap water, group B received 2 ml/kg body weight of soybean oil, group C received 2 ml/kg body weight of olive oil, while group D received 2 ml/kg body weight of margarine respectively, orally for 21 days. At the end of administration of the different dietary lipids, blood was collected by capillary action through ocular puncture of the eye into plain sample bottles. The blood sample was allowed to clot at room temperature and was centrifuged for 2000rpm for 10 minutes to obtain clear sera for hormonal assay.

Hormonal Assay: The sera obtained from the animal's blood sample were labeled and analyzed. Serum testosterone, progesterone, estradiol, prolactin and luteinizing hormone were assayed by the enzyme linked immunoassay (ELISA) method as described by Tietz [27] and Uotila *et al*. [28].

Statistical Analysis: The data obtained from all the groups were compiled and statistically analyzed and expressed as mean ± standard deviation. Differences between groups were compared using one way ANOVA, with $P<0.05$ considered significant.

RESULTS

From the chart above, the mean value of testosterone concentration level in group administered soybean oil (41.31 ± 3.18 ng/ml) showed a significant ($p<0.05$) decrease while the groups treated with olive oil (44.78 ± 2.18 ng/ml) and margarine (42.69 ± 3.75 ng/ml) showed a non-significant ($p>0.05$) decrease when compared to the mean value of the control (46.00 ± 2.39 ng/ml) group.

From the chart above, the groups administered soybean oil and olive oil with the mean values of (6.68 ± 0.42 ng/ml) and (6.72 ± 0.45 ng/ml) showed a non-significant ($p>0.05$) decrease relative to the control (6.47 ± 0.42 ng/ml) group, while the group administered margarine (7.75 ± 1.36 ng/ml) showed a non-significant ($p>0.05$) increase in comparison with the control group.

From Fig. 3 above, the mean value of the group administered soy oil (1.64 ± 0.28 pg/ml) and margarine (1.65 ± 0.10 pg/ml) showed a non-significant ($p>0.05$) increase as against the control (1.54 ± 0.32 pg/ml) group while the group administered olive oil (1.58 ± 0.12 pg/ml) showed a non-significant ($p>0.05$) difference when compared to the control group.

From the above chart, the mean value of the group administered olive oil (20.57 ± 15.00 mIU/mL) showed significant ($p<0.05$) increase accompanied by non-significant ($p>0.05$) difference in margarine (15.71 ± 6.70 mIU/mL) and soy oil (15.46 ± 1.76) treated groups relative to the control (15.18 ± 3.58 mIU/mL) group.

From the figure above, the groups administered olive oil (17.16 ± 10.81 mIU/mL) and soy oil (17.08±7.62 mIU/mL) showed a significant ($p<0.05$) increase when compared to the control (12.41 ± 4.44 mIU/mL) group while the group administered margarine showed a non-significant ($p>0.05$) increase in comparison with the control group.

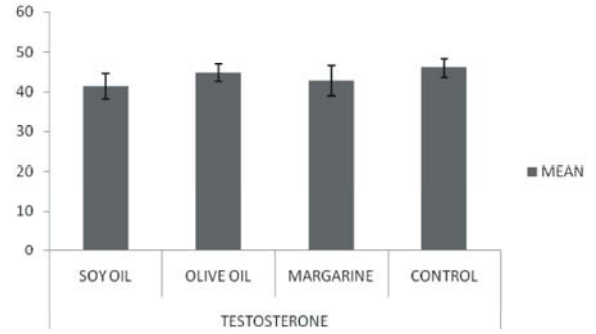

Fig. 1: Effect of dietary lipids on Testosterone

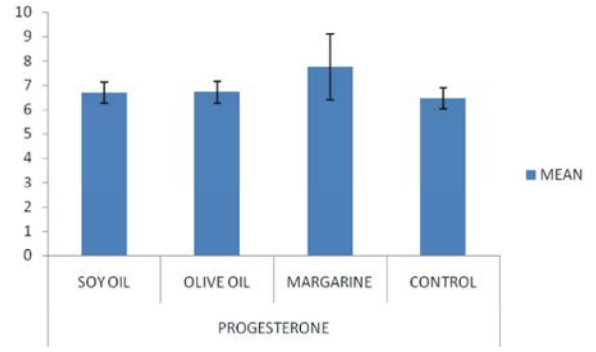

Fig. 2: Effect of dietary lipids on Progesterone

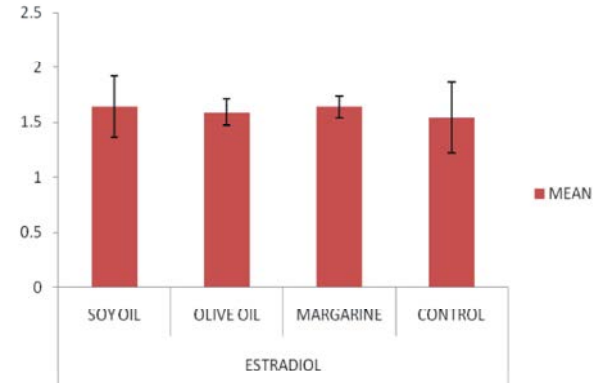

Fig. 3: Effect of dietary lipids on estradiol

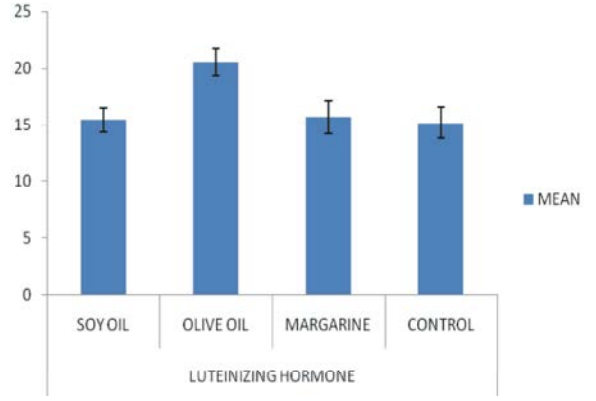

Fig. 4: Effect of dietary lipids on LH

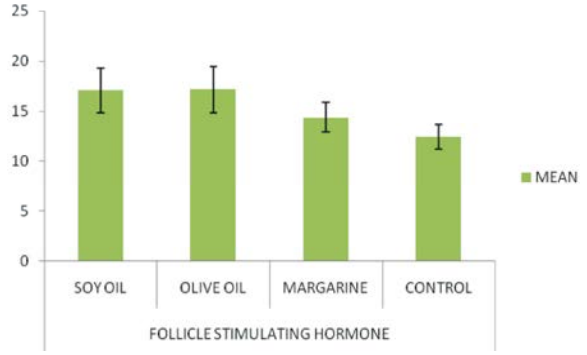

Fig. 414: Effect of dietary lipids on FSH

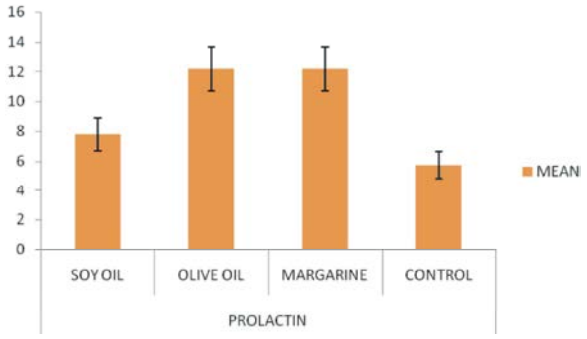

Fig. 5: Effect of dietary lipids on Prolactin concentration

From the chart above, the group administered olive oil (12.19 ± 9.70 ng/ml) and margarine (12.19 ± 6.84 ng/ml) showed a significant (p<0.05) increase accompanied by a non-significant (p>0.05) increase in the group administered soy oil (7.78 ± 3.41 ng/ml) relative to the control (5.71± 2.40 ng/ml) group.

DISCUSSION

Hormonal responses play a role in mediating the physiological and behavioural processes that influence human fertility [17- 19]. Much focus is on the hormone that secretes reproductive axis, how they are regulated and secreted [20]. The brain and pituitary coordinate and provide the central drive" to the reproductive axis throughout life, the brain is also the primary site where environmental factors that modulate reproductive function act. The region of the brain involved in the regulation of reproductive function as well as many of the bodies other basic homeostatic function is the hypothalamus. Decreased firing of gonadotropin releasing hormone neurons leads to less GnRH stimulation of pituitary luteinizing and follicle stimulating release and thus stimulation of ovarian and testicular function [21- 22].

In the present study, serum testosterone showed a significant (p<0.05) decrease in the group treated with soybean oil accompanied by a non-significant (p>0.05) decrease in the groups treated with olive oil and margarine respectively, relative to the control group. The marked decrease in testosterone level in group treated with soybean oil agrees with an earlier report of Staples *et al*. [23], who documented the decrease in testosterone level after consumption of dietary lipid. Steiner and Cameron [24] also suggested a possible anti-androgenic property of the treatment on the number of Leydic cells which is responsible for the manufacturing of testosterone. Progesterone and estradiol are among the most important sex hormones for implantation of the blastocyst and pregnancy maintenance [25]. In pregnant females, with a normal menstrual cycle, the progesterone level remains relatively constant throughout the follicular phase of the menstrual cycle and then increases rapidly following ovulation, while the estradiol secretion follows a cyclic biphasic pattern, with highest concentration found immediately prior to ovulation [2, 4 , 5]. Estradiol is mainly synthesized by the granulose and theca cells of the ovaries. Serum estradiol level is principally used to monitor the induction of ovulation and differential diagnosis of amenorrhea. From the results of this study, serum progesterone level was non-significantly (p>0.05) reduced in soy oil and olive oil treated groups accompanied by a non-significant (p>0.05) increase in margarine treated group in comparison with the control group. Furthermore, serum estradiol level showed an insignificant (p>0.05) increase in the groups treated with soybean oil and margarine whereas olive oil group showed an insignificant (p>0.05) difference relative to the control group, indicating that the hypothalamus-pituitary gonadal axis was not affected.

Luteinizing hormone is produced by gonadotrophic cells in the anterior pituitary gland. In females, an acute rise of luteinizing hormone (LH surge) triggers ovulation whereas in males, it stimulates Leydig cell production of testosterone [26]. In this study, serum luteinizing hormone level showed a significant (p<0.05) increase in the groups administered olive oil followed by a non-significant (p>0.05) difference in the groups administered soybean oil and margarine respectively, relative to the control group. It is known that luteinizing hormone stimulates ovulation growth of corpus luteum and progesterone release [28]. Therefore, LH acts to augment progesterone secretion by granulose cells, which stimulates FSH release at midcycle [13,14]. Moreover, the results obtained in the present

study is in line with those reported by Miller *et al.* [18] who documented that oleuropein aglycone in virgin olive oil is responsible for the increase in luteinizing hormone secretion through the enhancement of the pituitary gland by the increase in noradrenaline plasma level.

Follicle stimulating hormone is a glycoprotein produced in response to gonadotropin-releasing hormone [9]. The principal function of follicle stimulating hormone is to stimulate gametogenesis, follicular development in females and spermatogenesis in males [10,13]. In females, FSH acts on immature follicular cells of the ovary and induces development into mature follicle and oocyte capable of steroidogenesis. In the present study, serum FSH level in the groups treated with olive and soybean oil was significantly (p<0.05) increase accompanied by a non-significant (p>0.05) increase in those treated with margarine, relative to the control group. The significant increase in the level of follicle stimulating hormone may be due to ovarian steroidogenesis [16]. The increase in LH and FSH levels showed that the dietary lipids could stimulate ovulation in female rats. It may also stimulate hypothalamus-pituitary ovarian axis which is responsible for the synthesis and storage of gonadotrophins (LH and FSH), which play a major role as regulators of folliculogenesis [20].

Prolactin is a hormone that plays a role in fertility by inhibiting follicle stimulating hormone and gonadotropin releasing hormone (GnRH), the hormones that trigger ovulation and allow eggs to develop and mature. In the present study, prolactin level in the olive oil and margarine treated groups was significantly (p<0.05) increased accompanied by a non-significant (p>0.05) increase in the group treated with soybean oil, relative to the control group. High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle stimulating hormone and gonadotropin-releasing hormones (GnRH) [21] which are necessary for ovulation. Based on the results obtained in this study, it shows that the three different dietary lipids (soybean oil, olive oil and margarine) especially soybean oil and olive oil, which contained omega-3 and omega-6 PUFAs and MUFAs have strong capability of enhancing hormonal functions by stimulating hypothalamus-pituitary ovarian axis and subsequently the fertility in females. Hence, the consumption of these lipids could stimulate ovulation in female subjects and may be of help to treating ovulation disorders in females.

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