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المعلومات الشخصية

اسم الباحثة	نياف نضال كاظم الشمري
الكلية والقسم	كلية العلوم – قسم التقانة الإحيائية / جامعة النهرين
العنوان الدائم	بغداد – السيدية
البريد الالكتروني	niyaf_alshemmary@yahoo.com
الهاتف	+(٩٦٤) ٧٩٠٢٣٣٩٧٣٩
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The Effects of Some Fat Soluble Vitamins and Sunflower Oil on Reproductive Performance and The Level of Serum Total Cholesterol in Female Mice

A Thesis submitted to the Collage of Science Al-Nahrain University as Partial Fulfillment of The Requirements for the Degree of Master of Science in Biotechnology

By
Niyaf Nidhal Kadhem Al-Shemmary
B.Sc. ٢٠٠٢
(Al-Nahrain University)

Dr. Khulood Al-Sammaraei

Dr. Muhammad-Baqir M-R Fakhrildin

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Tho Al-ki'eda, ١٤٢٦

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ سُبْحَانَ الَّذِي خَلَقَ الْأَزْوَاجَ كُلَّهَا مِمَّا
تُنْبِتُ الْأَرْضُ وَمِنْ أَنْفُسِهِمْ وَمِمَّا لَا
يَعْلَمُونَ ﴾

صَدَقَ اللَّهُ الْعَظِيمُ

سورة يس

أيه ٣٦

الإهداء

غاية الحمد والشكر لولي التوفيق والفضل العظيم ربي

إلى كل ذرات التراب الغالية المخضبة بدماء الأبرياء وطني العراق

إلى من زرع الطموح ورغبتي أن أكون مثلهم أساتذتي

إلى من غرسني ولم يشهد أوان عطائي والدي

إلى من علمني المطاولة والصبر والإصرار على التفوق والدتي

إلى عضدي أخواي نورس ومحمد

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Summary

Antioxidants are chemical compounds that can delay the start or slow the rate of lipid oxidation reaction in food system. Antioxidants considered one of the best protection agents for the body against the side effects of free radicals, enhancing growth factor, improvement of fertility performance and reducing level of total serum cholesterol. Therefore, in this study was planned to investigate effects of orally intake, not exceeded the recommended daily allowance (RDA), of some antioxidants involving fat soluble vitamins (A and E) and sunflower oil, on the body weight, outcomes of *in vivo* and *in vitro* fertilization, level of total serum cholesterol and histological changes of uterine horns and ovaries of one hundred and eighty healthy female mice were divided into three major groups according to period of antioxidant administration involving 7, 14 and 28 weeks. Each major group was subdivided into five minor groups according to types of antioxidants involving vitamin A, vitamin E, vitamins A+E, positive control (sunflower oil) and negative control. Results of this study are summarized as fallow:

1. Body weight increased for mice treated with vitamin E for 7, 14 and 28 weeks, this group share the same elevation for mice treated with vitamins A+E treated for 14 weeks.
2. Hormonally stimulated-mice have higher absolute weights of reproductive organs than the natural cycle-mice. For the three treatment periods, administrations of different vitamins have better results than the negative control. While results recorded for absolute weights of ovaries and uterine horns for the group treated vitamins A+E for 14 weeks.

3. Treated groups with different antioxidants record better results in the pregnant weights and litter size than the negative control group for the three treatments periods. However, the best result was recorded for the outcome of *in vivo* fertilization for mice treated with vitamins A+E and vitamin E for 14 weeks.

4. Vitamins A+E-treated mice group show the best outcome of IVF, especially in the total oocytes collected from this group treated for 14 weeks, but the best percentages of IVM and IVF were recorded for mice group administered vitamins A+E for 7 weeks. While the lowest percentage of abnormal embryonic development was assessed for mice group administered vitamin E for 14 weeks.

5. Histological study appeared that the uterine horns and ovaries of hormonally stimulated groups treated with antioxidants have much better features in both longitudinal sections than the control groups for natural cycle- and/or hormonally stimulated-mice. These uterine features involve thickness of epithelial layer, diameter of uterine horns and uterine glands distributed along the uterine horns. Also, it was reported that hormonally-stimulated mice treated with vitamin E and vitamins A+E for the three treated periods were the best features. Moreover, best results for number and diameter of growing follicles Graafian follicles and corpus luteum were reported for hormonally stimulated-mice treated with vitamin A and vitamins A+E for 7 and 14 weeks, while, administration of vitamin E have best results for number and diameter of corpus luteum for the three treated periods.

6. Long-term treatment with antioxidants reduces the level of total serum cholesterol than the negative control group and the lowest level of total serum cholesterol was observed for the positive control group after 14weeks.

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List of abbreviations
AHD: Artery heart disease
AOW: Absolute Ovarian weight
AR: Acrosomal reaction
AUHW: Absolute uterine horns weight
AW: Absolute weight
C.L.: Corpus luteum
CC: Cumulus cells
DNA: Deoxy-ribonucleic acid
ET: Embryo transfer
G.F: Graafian follicles
Gr.F: Growing follicles
hCG: human chorionic gonadotrophin
HDL: High-density lipoprotein
hMG: human postmenopausal gonadotropin
IP: Intra peritoneal
IU: International unites
IVF: *In vitro* fertilization
IVM: *In vitro* maturation
LDL: Low-density lipoprotein
Pb: Polar body
RBP: Retinol binding protein
RDA: Recommended daily allowance
ROS: Reactive oxygen species
SOP: Superovulated program
TSC: Total serum cholesterol
ZP: Zona pellucida

CHAPTER ONE

Introduction

1.1. Introduction:

Antioxidants are wide range of chemical compounds occure naturally in plant or animal and could be manufactured. However, vitamins are powerful antioxidants like vitamins A, E and vitamin C (Jalal and Fuller, 1995; Anderson and Young, 2003). Also, many natural oils considered as powerful antioxidants like sunflower oil (Dorrell, 1981).

Vitamins A, E are fat-soluble and their absorption from the intestinal tract coupled with lipids (Borenstein *et al.,* 1988). These two fat soluble antioxidant vitamins wildly found in the healthy diet, however, rarely recorded cases for individual with malnutrition (Clarkson, 1995; Wildman and Medeiros, 2000). Vitamin E was first discovered in (1922), with experiments on rats and considered as antisterility factor (Bramley *et al.*, 2000). It was suggested that vitamin E has favorable effects on fertility and it's deficiency causes infertility in animals (Thiessen *et al.*, 1975). On the other hand, it enhances the fertility in human (Bayer, 1994). In addition, this vitamin reduces menopause symptoms (Perloff , 1989; Gozan, 1997). Vitamin E has protective, nutritional antioxidant function it is also performed and enhanced by other antioxidant and helps in maintaining the biological activity of vitamin A (Packer, 1992). Estrogen may reduce the effect of vitamin E (Traber and Ies, 1996). Increased free radical activity has been demonstrated to reduce male and female fertility and it has been suggested that antioxidants can be used to scavenge free radicals associated with subfertility and/or increased the total serum cholesterol (Crary and McCarty, 1984; Azen *et al.*, 1996).

Experimental studies have been shown that vitamin A can improve metabolic control and regulate sex hormones metabolism and secretion (Dudas, 1996; Bardanier *et al.,* 2001). Meanwhile this vitamin increases progesterone level slightly (Panth, *et al.*, 1991), at the same time it improves performance of reproductive organs (Lithgow and Politzer, 1977). Furthermore, it was suggested that vitamin A helps menorrhagical women and improves natural menstrual blood flow. Vitamin A is thought to play a key role in glycoprotein synthesis, which is in turn, are important for multiple cellular processes including communication, recognition, adhesion, aggregation, reproductive activities, bone development and immune system function (Al-Zuhairy, 1992).

On the other hand, vitamin A is used in skin, breast, prostate and uterine cancers treatment (Ballew *et al.,* 2001). Those two fat-soluble vitamins with antioxidant activity cause remarkable fall in total serum cholesterol, general fat accumulation and assist in healing and minimizing clotting (Wildman and Medeiros, 2000).

Sunflower oil was considered one of the edible oils and has no side effects and no therapeutic applications (U.S. Department of Agriculture, 1998). It was certified that this oil has powerful antioxidant activity due to it's high content in vitamin E, the most powerful antioxidant vitamin (National Academy of Sciences, 2000; NIH clinical center, 2001). Furthermore, sunflower oil was reported as one of the best natural source lowering cholesterol level (Morrison and Robertson, 1978; National Cholesterol Education Program, 1993).

1.2. Aim of the study:

The aim of the present study were to investigate effects of the fat-soluble antioxidants vitamins A and E separately and combined with the use of sunflower oil as edible oil, on female reproductive performance and total serum cholesterol level, in addition to the body weight through out different periods.

CHAPTER TWO

Literature Review

2.1. Mouse reproductive systems:

2.1.1. Female reproductive system:

Female reproductive system of the mouse consists of the ovaries, oviducts, uterine horns and vagina (Guyton, 1981). This reproductive system was based on the ovaries where female germ cells are grown and stored. The ovaries are small glands; located at the tips of the uterine horns. Vagina is the short grey tube lying dorsal to the urinary bladder and divides into two uterine horns extending toward the kidneys against the dorsal body wall (Vodopich and Moor, 1992). The coiled tubes between the ovaries and the uterine horns are the oviducts. Oviducts capture eggs produced by the ovaries and offer an environment to fertilize ova (Figure 1). One of the important functions of the female reproductive system is to receive male gametes and bring them to interact with female gametes to achieve fertilization (Lippold and Cogdell, 1991)

2.1.1.1. Oogensis:

A process undergoes mitotic division started with ovarian primary oocyte result only one functional cell so called ovum (Browder, 1985). The primitive ovum or oogonium (has a diploid number of chromosomes; 2n (Seeley *et al.,* 1996). It divides by mitotic divisions to form primary oocytes the primary oocytes divides by meiotic divisions into functional secondary oocytes (with haploid number of chromosomes; 1n) and non-functional first polar body (1^{st} Pb). The secondary oocytes enter the mitotic division and forms ovum and second polar body (Blandau, 1980). This process will be summarize as follow:

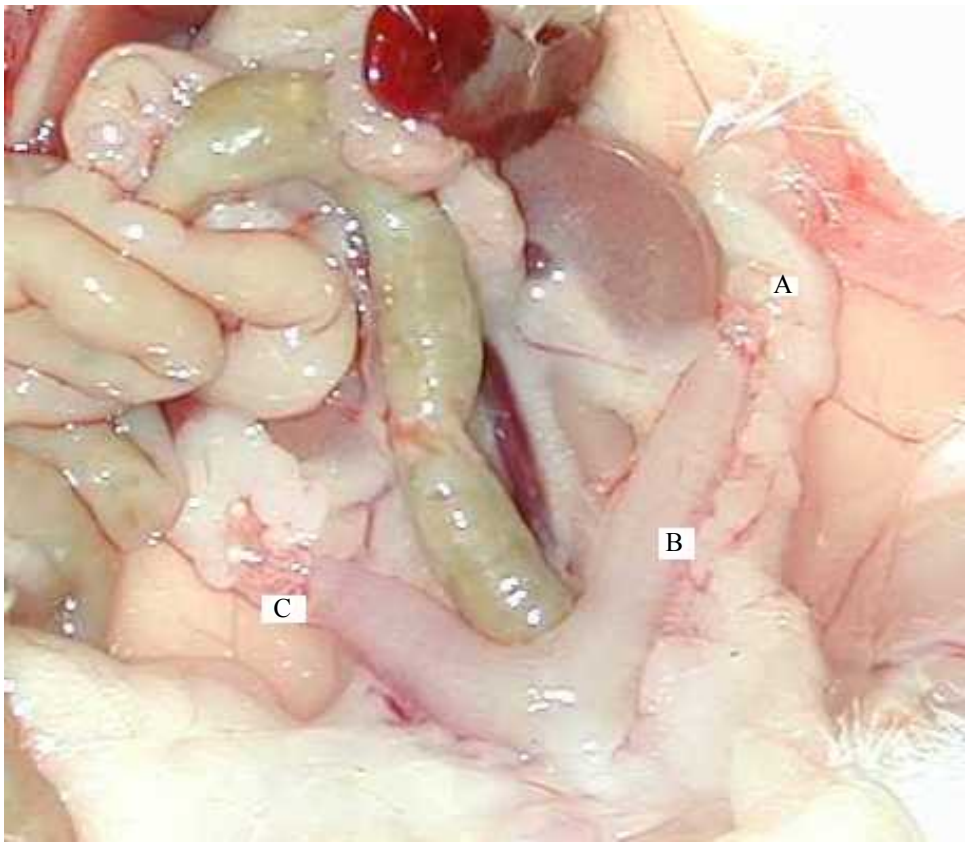


Figure 1: Reproductive system of mouse female; (A: Ovary, B: Uterine horn, C: Fallopian tube).

The primitive germ cell oogonia developed in the embryonic phase and further development during the fetal phase. At puberty, primary oocytes undergo the first meiotic division.

Whereas all eggs present at birth are primary oocytes cells at meiotic arrest and complete the 1st. meiotic division were produced secondary oocyte and 1st polar body were formed at last , second meiotic division occurs after ovulation if secondary oocyte is activated by penetration of sepermatozoon and extrusion of 2nd Polar body (Vodopich and Mooro, 1992).

2.1.1.2. Ovulation:

It is the process of releasing an ovum into abdominal cavity after the rupture of Graafian follicle. This is due to activity of proteolytic enzymes. Follicular growth was regulated by pituitary gonadotropins (Guyton, 1981). Following ovulation, the Graafian follicle collapses and blood within it forms a clot called the corpus hemorhagicum. The remaining follicular cells eventually absorb the clot. At the same time, the follicular cells enlarge, change character and form the corpus luteum as a yellowish body (Blandau, 1980). A matured ovum, released during ovulation, characterized as a large spherical cell having a thick, hyaline, transparent, mucoprotein and non-cellular investment so-called zona pellucida, which surrounds the vitelline membrane. The space between vitelline membrane and zona pellucida called perivitelline space. On ovulation, ovum released along with surrounding cumulus cells (Lippold and Cogdell, 1991). The nucleus of immature ovum is large, round and usually found eccentrically with a well-marked nucleolus. The fully mature ovum has only half the number of chromosomes because of meiosis during the maturational divisions (Browder, 1985).

2.1.1.3. Estrous (estrus) cycle:

During the reproductive life span, the mammalian female exhibit cyclic changes in the ovary, which may lead periodically to ovulation (Maller, 1985). This activity affecting the entire reproductive tract and other accessory tissues and depending on endocrine factors and in non-human primates referred to as the estrus cycle. Certain mammals such as rat, mouse, or cow reproduction is possible throughout the entire year (polyestrous)

(Guyton, 1981). Ovulation occurs at a well-defined period and may bring with it changes in the reproductive tract which permit implantation of the fertilized egg. The ovarian hormones affect these changes, which in turn are under the control of the pituitary gland. Marked changes occur in the vaginal epithelium during both the estrous and menstrual cycles, these changes have been correlated with changes in the ovary and uterus, and some instances are good indicators of the stage of the cycle (Lippold and Cogdell, 1991). In the mouse, the cyclic activity are 4-5 days as a cycle length and 10 hours duration of estrus and 2-3 hours after onset of estrus that was the ovulation time (Browder, 1985).

2.1.2. Male reproductive system:

Male reproductive system of the mouse consists of the primary reproductive organs, the testis and the accessory reproductive organs, including the scrotal sac, epididymes, tubular vas deferens, seminal vesicles, urethra, prostate gland, bulbourethral glands and penis (Figure 2) (Seeley *et al*., 1996). The male testicle manufactures and stores the male gametes and then delivers them into the female vagina. Sperm cells are transported from the testis, (in which the sperm cells develop) to the epididymis and then through the vas deferens into the pelvic cavity (Wassarman, 1987). Epididymis is the coiled tube on the surface of the testis in which the sperm cells are stored, which lies on the external surface of each testis just before the vas deferens enters the prostate gland below the bladder. Vas deferens increases in

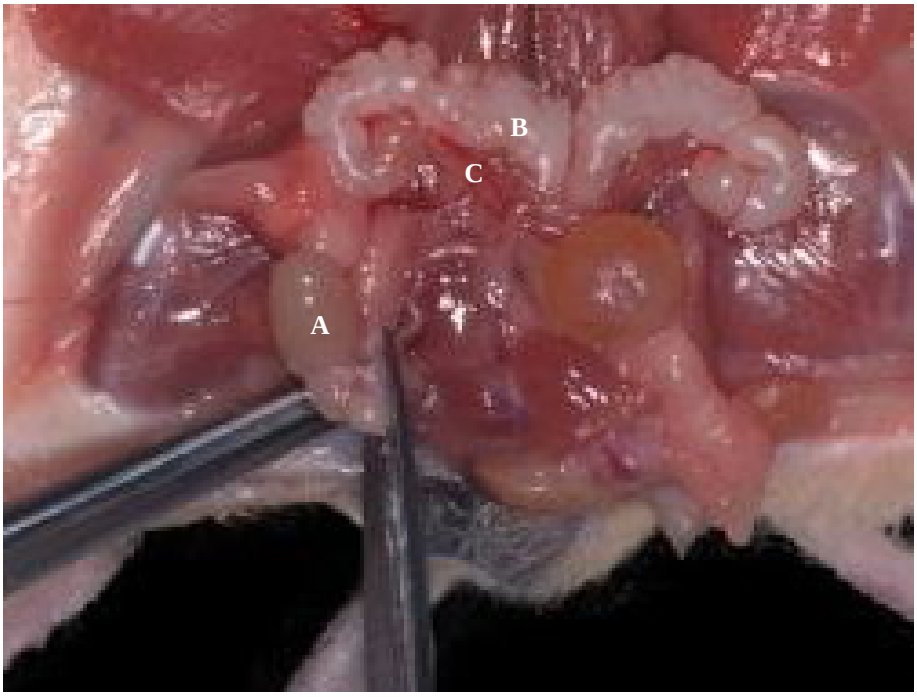


Figure 2: Reproductive system of mouse male; (A: Testes, B: seminal vesicles, C: tubular vas deferens)

diameter to become the ampulla of the vas deferens. Anatomically, seminal vesicles appear as brown glands located to the right and to the left of the urinary bladder (Vodopich and Moore, 1992). A short duct of seminal vesicles joins the ampulla of the vas deferens to form the ejaculatory duct at the prostate, which the projects through the prostate gland and empties into the urethra which carries sperm through the penis to the outside (Lippold and Cogdell, 1991).

2.2. Antioxidants:

Chemical compounds that can delay the start or slow the rate of lipid oxidation reaction in food systems, therefore, the main function of them is to minimize lipid oxidation (Dimascio *et al.*, 1989). Antioxidant defined as scavengers of free radicals in human body tissue (Block *et al.*, 2001). If a compound inhibits the

formation of free alkyl radicals, in the initiation step, or if the chemical compound interrupts the propagation of the free radical chain, the initiation of free radical formation can be delay by the use of metal chelating agents, singlet oxygen inhibitors and peroxide stabilizers (Crary and McCarty, 1984; Mammoto *et al.*, 1996).

The propagation of free radical chain reaction could be minimizing by the donation of hydrogen from the antioxidants and the metal chelating agents (Clarkson, 1995).

2.2.1. Characteristics of antioxidants:

The major antioxidants currently used in foods are monohydroxy or polyhydroxy phenol compounds with various ring substitutions (Block *et al.,* 2001). These compounds have low activation energy to donate hydrogen. The resulting antioxidant free radical complex does not initiate another free radical due to the stabilization of delocalization of radical electron. The resulting antioxidant free radical complex was not subject to rapid oxidation due to its stability (Halliwell, 1997). The antioxidant free radicals complex can also react with lipid free radicals to form stable complex compounds (Mammoto *et al.*, 1996). Synergism occurs when mixtures of antioxidants produce more pronounced activity than the sum of the activities of the individual antioxidants when used separately (Halliwell, 1996). To have maximum efficiency, primary antioxidants are often used in combination with other phenolic antioxidants, or with, various metal chelating agents (Kamal-Eldin *et al.*, 1996).

Different antioxidants show substantially different antioxidant effectiveness in different fats, oils and food systems

due to different molecular structures (Azen *et al.*, 1996). So when choosing an antioxidant it should be safe enough, most effective, off-odor, off-color, with stability to pH and food processing, available , and non-absorbable if possible (Halliwell and Gulteridge, 1989). Indeed antioxidants have no harmful physiological effects, effective in low concentration, should be fat-soluble, and carry-through effect (no destruction during processing) and economically acceptable (Crary and McCarty, 1984).

Antioxidants have the ability to destroy the free radicals that damage cells, promote the growth of healthy cells, protect cells against premature, abnormal aging , help fight age-related macular degeneration, and provide excellent support for the body's immune system making it an effective disease preventative (Clarkson, 1995).

2.2.2. Antioxidant vitamins and female fertility:

It was reported that the vitamins A and E appear have powerful antioxidant properties (Anderson and Young, 2003). Moreover, their effects on male and female fertility were mentioned in details (Clarkson, 1995). For example, women complaining from excessive bleeding which leds to anemia, cramps and lastly, debilitating. Although, no other problems are associated with a woman's cycles that can't be corrected by simply getting proper amounts and dosages of vitamins A and E. Studies have shown that women with menorrhagia (excessive bleeding) were invariably deficient in vitamin A (Bendich and Machlin, 1988, Bendich and Langseth, 1989). Without proper amounts of vitamin A, the gonads cannot manufacture the sex hormones, and

then release them into the bloodstream of the male or female to regulate their sexual desires and abilities (Dudas, 1996). Vitamin A increases progesterone levels (Panth *et al.* 1991).

There is much more evidence that the vitamin A improve the sexual health and performance. Many studies with vitamin A have proved its efficacy in fighting cancer. Moreover, taking extra vitamin A may ward off cancers of the cervix and prostate gland (Jill and Manzoni, 2001). According to results of experimental researches, vitamin E deficiency leads to infertility in animals (Thiessen *et al*., 1975). However, a dose of 100 to 200 IU daily, it has been shown to increase fertility in humans (Bayer, 1994). Vitamin E appears to be helpful in the reduction of menopause symptoms (Perloff, 1989; Gozan, 1992). Vitamin A deficiency is quite common in women with menorrhagia. It was mentioned that the 25,000 IU taken twice daily for two weeks causes' improvement and sometimes even complete return to a normal menstrual blood flow (Lithgow and Politzer, 1977). However, low levels of vitamin A also appear to make in utero transmission more likely. However, since high levels of vitamin A supplementation can be dangerous during pregnancy, (Semba *et al.* 1994).

Supplemental estrogen or estrogen imbalance in women increases the need for vitamin E (Bayer R, 1994) ; Estrogen may decrease the effect of vitamin E, so more is needed when estrogen therapy is used. Although vitamin E was first discovered as the fertility factor, or at least the antisterility agent, nutrient, there is no clear evidence that it enhances fertility if there is not a specific deficiency prior to its use. Many people, especially men, take vitamin E with some claimed success concerning sexuality and vitality. Much of this effect, however, may be due to the

antioxidant function and improved circulation and oxygenation (Groff *et al*., 1995). Vitamin E may be very helpful to women complaining from menstrual pains, as well as general relief from various menstrual disorders. Many problems of menopause, such as headaches, hot flashes, or vaginal itching due to dryness, may be reduced with the use of supplemental vitamin E. When birth control pills are used, the tocopherols may help protect the body from their possible side effects (Halliwell and Gulteridge, 1989).

Vitamin E has been used both topically and orally with some success in the treatment of fibrocystic breast disease, or cystic mastitis, likely due to its protective mechanisms against estrogen,which seems to potential for this disease (Chan, 1993

2.3. Free radicals:

Free radicals are molecules with an unpaired electron (Halliwell and Gulteridge, 1989), unstable molecule that steals an electron from a stable molecule in order to satisfy its need for repair (Halliwell, 1996). In doing so, this free radical destabilizes the stable molecule and creates another free radical in a vicious chain reaction of cellular destruction (Diplock *et al*., 1998).

A single free radical can cause damage to millions of other molecular destruction is continually occurring in the body (Jacob and Burri, 1996). Free radicals attack the body from many different environmental sources every day some of which are: alcohol, tobacco, prescription, drug, smoked and barbecued food, harmful chemicals and food additives, sun bathing and pollutants in the air. They assault the cells large enzyme complexes (Crary and McCarty, 1984). Increase in age mean increase much free radical

(Yu, 1994). Scientists have determined that large amounts of free radicals accumulated in the body may significantly shorten the life span (Block *et al.,* 2001).

Normal metabolism produces a small amount of those free radicals (Clarkson, 1995). Oxidative phosphorylation, for example involves reduction of molecular oxygen by stepwise addition of electrons (Collier *et al.,* 1990). Under normal conditions, ninety eight percent of the oxygen was reduced to water, but two to five percent of the reduced oxygen enters the univalent pathway, which produces by-products called reactive oxygen species (ROS) (Machlia and Bendich, 1987; Sies, 1991). Most of the ROS are taken care of by superoxide dismutase, catalase and glutathione peroxidase (Halliwe, 1997).

2.4. Vitamins:

Vitamins are organic substances, not synthesized within the body it is essential in small amount for the maintenance of normal metabolic functions. In 1911, first termed vitamin when it called (vital amine) but not all vitamins are amines like A, C, D, E, K and insitol that lack nitrogen function of any type (Heseker, 1992).

Vitamins may be used as special dietary supplements or as drugs if they are taken to treat a condition of vitamins deficiency or to prevent imminent development of a disease (Sies, 1997; U.S. Department of Agriculture, 2000). Risks to health associated with daily ingestion of unnecessary vitamins are undoubtedly less than with over ingestion of caloric foods (Borenstein *et al.,* 1988). However, the pharmacist and other health professionals must be alert to the need for detailed diagnostic assessment if an actual deficiency is suspected. The actual dietary needs depend on a

number of variables including age, illness, sexes, stress and weight (Ross, 1999).

2.4.1. Fat-soluble vitamins

Vitamins A, E, D and K are fat soluble, their absorption from the intestinal tract is associated with that of lipids (Borenstein *et al.*, 1988).

2.4.1.1. Vitamin A:

Vitamin A is a yellow to red oily liquid that may solidify. It may be nearly odorless or may have a slightly fishy odor. It has no rancid odor or taste (Anderson and Young, 2003). It might dilute with edible oils, or it might incorporate in solid, edible carriers (Ross, 1999). Retinol is the major natural form of the vitamins, all-trans retinol are the major commercial forms of vitamin A. Retinol is readily absorbed (80-90) % from the normal intestinal tract and stored in body tissues especially the liver (Bendich and Langseth, 1989).

The usual use recommended daily allowance (RDA) of vitamin A for adults and children over 4 years 5000 units (some times expressed as 1000 retinol equivalents). However, RDA for infants are 1500 units while for children under the age 4 RDA taken 2500 units, pregnant and lactating women 8000 units [1 unit is equal to 0.3 microgram of retinol] (Olson, 1987). Fish liver oils are the richest known natural sources of the vitamin and formerly were its primary commercial sources (James, 1998).

Two main sources of vitamin A are known, animal origins (heart, liver, kidney, eggs, dairy products and fish) and plant origin (carotenoids in both carrots and green leafy vegetables) (Guthrie,

1995). Most retinoids are soluble in organic solvent and fat however, oxidation and polymerization are all detrimental to retinoid; therefore, the compounds must be protected from light oxygen and high temperature (Bendich and Langseth, 1989).

Dietary function of vitamin A has been shown to possibly have some antioxidant characteristics. In recent years, the carotenoids such as β-carotene have received more attention from the scientific community because of the harmful role, they may play as pro-oxidants (Volpe, 2000). Retinol, the active form of vitamin A, rarely found in foods, instead, precursors to retinol, fatty acid retinyl esters found in the human diet. The esters commonly found in foods of animal origin (National Academy Press, 2001). In addition to egg yolks, liver, fish oil, whole milk and butter. The wide variety of vitamin A precursors allows for adequate amounts of the vitamin A in all diet types (Ballew *et al., 2001*)

2.4.1.1.I. Vitamin A chemical structure:

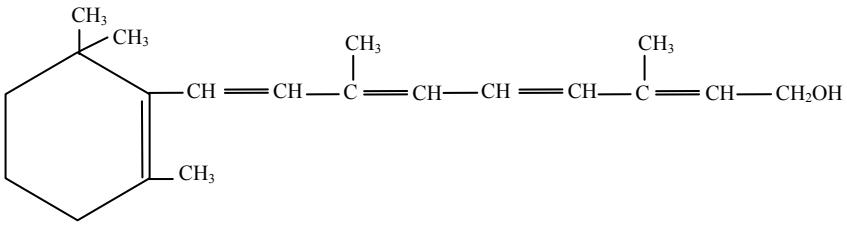


Figure 3: Chemical structure of Vitamin A ($C_{20}H_{26}OH$) (Rassam, 1987).

2.4.1.1.II. Metabolism" absorption and bioavailability"

Seventy to ninety percent of vitamin A within the constituent of the diet adsorbed in the intestine (Guthrie, 1995). The efficiency of absorption for vitamin A continues to be high (60-80%) as intake continues to increase greater then 90% of the retinol store within the body (Institute of Medicine Food and Nutrition Board 1999). Therefore, absorption of vitamin A is very rapid, with maximum absorption occurring two to six hours after digestion (Anderson and Young, 2003). Within the intestinal lumen, the vitamin A incorporated into a micelle and absorbed across the brush border into the cells. Then the precursors of vitamin A (carotenoids) converted to active forms of the vitamin, the newly formed products and additional precursors packaged into chylomicrons and transported throughout small intestine to all over the body (Garry *et al.*, 1987).

2.4.1.1.III. Physiological role:

Vitamin A as retinoic acid acts as a hormone. Retinoic acid first binds to retinoic acid receptors, then interact with specific nucleotide sequences of DNA. The interaction directly affects gene expression and transcription, which in turn control cellular

development and body function (Groff *et al.*, 1995). Vitamin A thought to play a key role in glycoprotein synthesis. Once formed, glycoproteins are important in multiple cellular processes including communication, recognition, adhesion, and aggregation, reproductive processes, bone development, along with maintenance, and immune system function (Erdman *et al.,* 1988).

The role of the retinoid in epithelial cell formation is very important for the treatment of skin cancer, acne, and acne - related diseases (Guthrie, 1995). However, β-carotene has been noted as having pro-oxidant properties. Moreover, vitamin A is help to repair the damaged tissue and therefore may be beneficial in counter-acting the impact free radicals damage (Groff *et al*., 1995).

2.4.1.1.IV. Deficiency and Toxicity:

Deficiency of vitamin A is very rare (Anderson and Young, 2003). Unless confounding malabsorption conditions such as: steatorrhea or diseases of the liver, pancreas and gallbladder are present (Viteri, 1988). In contrast, vitamin A deficiency is prominent in young children (<5 year old) living in third world countries (Garry *et al.,* 1987). At birth, neonates with low plasma vitamin A content, the levels corrected with a diet sufficient in vitamin A (Miller and Hayen, 1982). Symptoms of vitamin A deficiency include metaplasia, poor growth, xerophthalmia and keratinization of epithelial cells resulting in a loss of differentiation (Bendich and Langseth, 1989).

If vitamin A deficiency has not been chronic, it may lead to permanent debilitation. However, the symptoms can often be reversed through supplementation (Ross, 1999).

Toxicity caused by excessive amount of vitamin A taken in one dose or it may be caused by long treatment with the vitamin A which could show accumulative effects and toxicity condition would appear when the use of acne medicines (i.e. Acutane) has led to birth defects and even death (Argonz and Abinzano, 1960). In adults, a condition known as hypervitaminosis exhibits itself after chronic ingestion of the vitamin in doses that are ten times the RDA, symptoms of vitamin toxicity includes anorexia, headache, bone and muscle pain, vomiting, alopecia, liver damage, and coma. These symptoms slowly reside as vitamin A intake levels are reduced (Groff *et al.*, 1995). Researchers believe that the presentation of unbound retinol to the cell is a major factor in toxicity, excessive intakes of vitamin A saturate retinol binding protein (RBP) and instead of retinol being transferred bound to RBP, it is transferred to the tissue via plasma lipoproteins (Al-Senaidy, 2000). When retinol reaches the tissue by a carrier other than RBP it hypothesized that the retinol is released and causes toxic side effects (Bendich and Langseth, 1989).

2.4.1.2. Vitamin E:

Vitamin E was discovered in 1922 with experiments on rats, when fed a purified diet devoid of vitamin E, the rats became infertile. Wheat germ oil added to their diet restored their fertility (Bramley *et al.*, 2000). Later, the oil-based substance was isolated and called the "antisterility" vitamin. The origin of word tocopherol is (Tokos and phero are the Greek words and represent "offspring" and "to bear"; respectively, so tocopherol literally means, "To bear children.") (Guthrie, 1995). There is no clear deficiency disease in human beings. Vitamin E is well accepted as

an essential vitamin (Traber and Ies, 1996; Ford and Sowell, 1999). Vitamin E (Tocopherol) is light yellow oil, a fat-soluble vitamin, found in nature. That is actually a family of compounds, the tocopherols , α-tocopherol is the most common and the most active of the seven currently described forms-alpha, beta, gamma, delta, epsilon, and zeta (Anderson and Young, 2003). Although α-tocopherol, basically it is stable in heat and acids (Schudel *et al.*, 1972). While other forms, are lost in heat, with storage, freezing or when oxidized by exposure to the air (Erdman *et al.*, 1988).

All forms of vitamin E is slightly unstable in alkali and readily used up when in contact with polyunsaturated oils or rancid fats and oils, which are protected from oxidative destruction by vitamin E (Ford and Sowell, 1999). The primary and important function of vitamin E is an antioxidant. However, this protective, nutritional antioxidant function also performed and enhanced by other antioxidants (Chan, 1993).

Without vitamin E, cell membrane, active enzyme sites and DNA are less protected from free radical damage (Sue, 1995). More specifically, vitamin E as an antioxidant helps to stabilize cell membrane and protect the tissues of the skin, eyes, liver, breast, and testis, which are more sensitive to oxidation (McCay and King, 1980). It protects the lungs from oxidative damage by environmental substances. Vitamin E helps in maintaining the biological activity of vitamin A (Bramley *et al.*, 2000). Sources of vitamin E, as its various tocopherol forms, are found in both plant and animal foods. In general, the animal sources of vitamin E are poor, with some being found in butter, egg yolk, milk fat and liver (Guthrie, 1995). The best sources of vitamin E are the vegetable and seed or nut oils. It was first isolated from wheat germ oil,

which is still a commonly used, rich source of vitamin E (Green, 1972).

The oil component of all grains, seeds and nuts contain tocopherol. The protective covering of germ part of the grains that contains the vitamin E, and this is lost easily in the milling of flour or in the refinement of grains. Sunflower oil is one of the best sources, with about 90 percent of the vitamin E being the alpha variety, meanwhile corn oil has only about 10 percent of α-tocopherol (Cosowsky *et al*., 1972). Some other foods that contain significant amounts of vitamin E are soybeans, some margarine and shortenings made from vegetable oils, and a few vegetables, such as uncooked green peas, spinach, asparagus, kale, cucumber, tomato and celery also have a little (Bramley *et al.,* 2000).

Vitamin E was thought to raise high density lipid (HDL)"good" cholesterol levels, especially when they were low. Vitamins A and E together can help to decrease cholesterol and general fat accumulation (Asherio *et al.*, 1992). To assist in healing and minimize clotting, tocopherol is a useful nutrient before and after surgery, but is limited to dosages of 200–300 IUs per day, in spite of higher amounts may actually suppress the healing process (Azzi *et al.,* 2000).

2.4.1.2.I. Absorption and metabolism:

Vitamin E is absorbed from the intestines, along with fat and bile salts, first into the lymph and then into the blood, which carries it to the liver to be used or stored. Vitamin E is not stored in the body as effectively as the other fat-soluble vitamins A, D and K (Anderson and Young, 2003). Over half of any excesses may be lost in the feces, but some vitamin E is stored in the fatty tissues and the liver and to a lesser degree in the heart, muscles, testes, uterus, adrenal and pituitary glands and in the blood. Vitamin E is partially absorbed through the skin when used as an ointment or oil application (Bendich and Machlin, 1988).

2.4.1.2.II. Vitamin E as antioxidant:

Vitamin E protects the unsaturated fatty acids in the body and prevents the oxidation of some hormones, such as those released from the pituitary and adrenal glands. Free radical formation and oxidation are tied to cancer development, so the family of nutritional antioxidants including vitamin E may help in preventing tumor growth (Chan, 1993).

Vitamin E works as an electron receptor in the breaking of radical chain reactions. However, it is present in very low concentrations in the plasma membranes and lipoproteins (National Academic Press, 2000). One hypothesis is that vitamin E acts as a temporary store for the electron until other species can accept the electron and reduce vitamin E back to its original form. The species that oxidize the reacted vitamin E may fluctuate in concentration and only when all vitamin E is used up oxidation damage will occur (Packer *et al.*, 1992). If the amount of vitamin E in the plasma membrane and low-density lipid (LDL) were

increased, the amount of protection from oxidation would also increase. With reduced oxidation, less oxidized LDL would form and less damage would be occured (Kamal-Eldin and Appelqvist, 1996). Relation was found between plasma vitamin E levels and ischemic heart disease than blood pressure or cholesterol (Azzi *et al.,* 2000). Vitamin E should help to prevent oxidation of LDL, and therefore slow down the formation of early lesions. There is a natural line of defense against oxidation. However, vitamin E was proposed to be the main chain-breaking antioxidant in the lipid membrane of tissues and in LDL (Erdman *et al.,* 1988). 500 IU of vitamin E/day it may help but will not hurt (Simons *et al*. 1996).

2.4.1.2.III. Chemical structure of vitamin E:

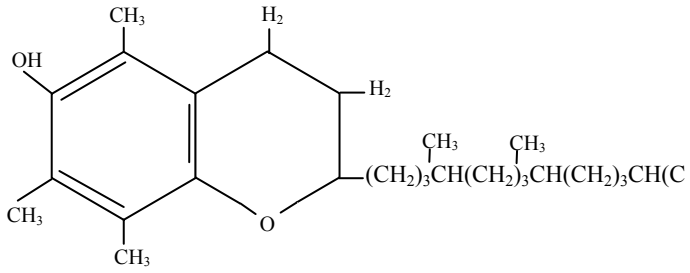


Figure 4: Chemical structure of α-tocopherol (Al-Zuhairy, 1992)

2.4.1.2.IV. Deficiency and toxicity:

Vitamin E is not stored as readily as are the other fat-soluble vitamins. Excess intake is usually eliminated in the urine and feces, and most doses clear the body within a few days. For these reasons, toxicity from vitamin E use is unlikely (Anderson and Young, 2003). However, smaller doses seem to be immune supportive (Azzi *et al.,* 2000).

Vitamin E deficiency is fairly rare (Miller and Hayen, 1982). Infertility as an effect of vitamin E deficiency has not been revealed as clearly in humans as it was in the rat study (Guthrie, 1995). It is likely that vitamin E deficiency is simply more difficult to diagnose symptomatically because of its wide range of effects on the nervous, reproductive, muscular and circulatory systems and because other nutrients may mask vitamin E deficiencies (Bendich and Machlin, 1988). However, biochemically, low levels of vitamin E can be measured in the blood and have been seen in such conditions as acne, anemia, infections, some cancers, periodontal disease, cholesterol gallstones, neuromuscular diseases, and dementias such as Alzheimer's disease (Ingold *et al.,* 1987).

Deficiencies are more of a concern in premature babies, since there is no maternal-fetal vitamin E transfer (Bramley *et al.*, 2000). Deficiency of vitamin E is also more likely in adults with gastrointestinal disease, with poor fat digestion and metabolism, or with pancreatic insufficiency (Ford and Sowell, 1999). The first sign of vitamin E deficiency may be loss of red blood cells due to fragility caused by the loss of cell membrane protection. Oxidized polyunsaturated fatty acids may also weaken the red blood cell membranes and cause rupture (Erdman *et al.,* 1988). Men with vitamin E deficiency may have changes in the testicular tissue (Anderson and Young, 2003). The amount required of vitamin E depends upon body size and the amount of polyunsaturated fats in the diet, since vitamin E is needed to protect these fats from oxidation. More is needed when any refined oils, fried foods, or rancid oils are consumed. Even though the RDA for vitamin E is really quite low, many people do not consume this in their diet alone (Bendich and Machlin, 1988). Vitamin E oil is taken ideally in the morning before breakfast or at night before bed. It can also be taken after meals containing some fat. Approximately 400–600 IUs is used preventively, whereas for therapeutic effects, an amount between 800–1600 IUs daily is suggested. With therapeutic uses of vitamin E, it is best to start with a low level and gradually increase it (Institute of Medicine Food and Nutrition Board, 1999). Levels over 1600 IUs / day are not recommended unless there is close medical supervision (Azzi *et al.,* 2000).

2.5. Sunflower oil:

Sunflower oil is the fatty oil (fixed oil) of *Helianthus annuus*, which is recovered from the fruits (excluding the shell), by cold pressing (expression). The mother tincture is extracted from the Jerusalem artichokes *Helianthus tuberosus*. The world's second most important source of edible oil (Dorrell, 1981). Sunflower oil is used for cooking, margarine, salad dressings, lubrication, soaps, and illumination (Duke and Wain, 1981). According to (Insel *et al.,* 2001), health risks or side effects following the proper administration of designated therapeutic dosages are not recorded. It is used as an inactive ingredient in pharmaceutical preparations (NIH Clinical Center, 2001). Sunflower oil should be kept protected from light, in tightly sealed containers. Oils from different deliveries should not be mixed (Dorrell, 1981). It was suggested that amount 2 tablespoons daily is enough to maintain proper function of body (Anderson and Young, 2002).

2.5.1. Physical and chemical properties:

Sunflower oil liquid as its physical state, color vary from yellow to green, have a slight odor, insoluble in water. Sunflower oil is high in vitamin E, one of the most powerful antioxidants in nature, especially in its natural form (U.S. Department of agriculture; U.S. Department of health and human services, 2000). It has also a very light-clean taste. Sunflower oil lowers serum cholesterol, promotes HDL lipid production and reduces the risk of vascular diseases (Elson, 1992). Heating oils like sunflower oil, rich in polyunsaturated, and refining them without care creates trans-fatty acids which is in turn decrease the HDL lipids in the body (Page, 1981).

2.6. Cholesterol:

In indispensable structural and metabolic components of all animal cells and the most important sterols found in such cells rather than plant cells (Stryer, 1995). Cholesterol binds with long unsaturated fatty acids in the blood and formed esters, and then transferred through blood via lipoproteins (Killian *et al.,* 1989).

2.6.1. Cholesterol occurrences:

Cholesterol found almost in animal cells and especially in cells of certain organs like liver, kidneys and brain in a level of 17% of brain dry weight, found basically in egg yolk, butter and cheese (Behrman and Aten, 1991). Cholesterol level in the blood reaches (150-250 mg/100ml). The liver could synthesized cholesterol in a range of (1-2) gm/day and cholesterol excluded out of body via feces in a range of (0.2-0.8) gm/day and (0.1-0.3) mg/day (Anderson and Young, 2002). After digestion lipoproteins, cholesterol results and only 50% of the cholesterol are taken up, the rest being lost in the feces (Al-Zuhairy, 1992).

2.6.2. Cholesterol function:

Cholesterol play an important anabolic role in which 80 % of it transformed to bile acids especially cholic acid, and small amount of cholesterol used by adrenal gland to form adrenal hormones, used by ovaries to form estrogen hormones and by testis to form testosterone hormones . Part of cholesterol transformed to 7-dehydrocholesterol which is considered as a precursor of vitamin D_3 (Stryer, 1995). Morethan one causes lead to the same condition called Atherosclerosis which in turn causes artery heart disease (AHD) (Jalal and fuller, 1995).

2.6.3. Chemical structure of cholesterol

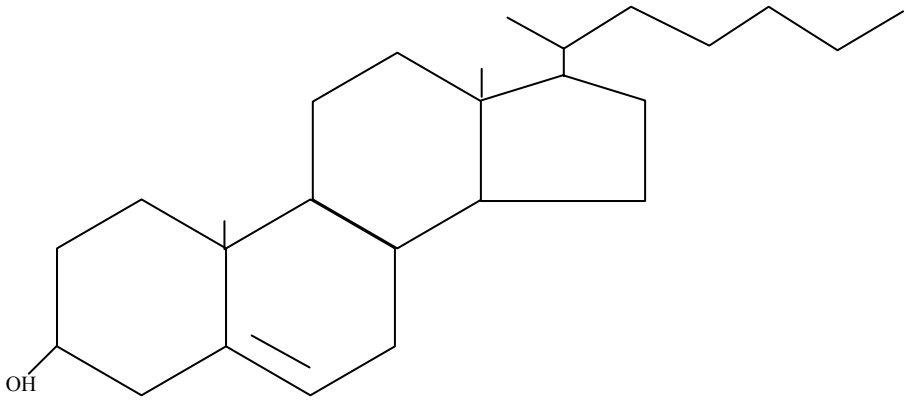


Figure 5: Chemical structure of Cholesterol (Al-Zuhairy, 1992)

2.7. *In vitro* Fertilization

Many of the major advances in mammalian fertilization and early embryogenesis are due to the relative ease with which it is now possible to achieve *in vitro* fertilization (Anderiesz *et al.*, 2000). *In vitro* fertilization is an alternative method for natural fertilization. This technique involved extracorporeal union of gametes, omitting the need for the uterine horn as a site of fertilization in animals (Bavister, 1981). Further, it has a clinical application in the field of infertility (Dukelow *et al.*, 1983). *In vitro* fertilization in animal species has been possible for the past 25 years (Blandau, 1980).

Methodology of IVF program includes ovulation induction, ova recovery, sperm suspension and preincubated for *in vitro* capacitaion, appropriate condition for penetration of the egg by the sperm, embryo culture and embryo transferant laboratory (Bavister, 1981). The fertilization rate ranged between (60-90) percent (Bavister, 1981; Yang and Yanagimachi, 1989).

Superovulation program (SOP) was considered one of the most important factors for successful IVF technique in the mammalian species and the first step in this technique (Edwards *et al.,* 1984). Additionaly, a variety of culture media were used in a different laboratories for *in vitro* oocyte maturation (Anderiesz *et al.,* 2000), as well as for preparation of sperm and oocytes for IVF (Calvo *et al.,*1993).

The process of maturation encompasses a complex series of molecular and structural events, culminating in the arrest of the oocyte.

Chromosomes on the metaphase-Π plate in anticipation of sperm penetration and activation for fertilization (Osborn, 1993). Nuclear maturation, the resumption of meiosis and completion of the 1st meiotic division may be occurs *in vitro* for all species studied as mentioned by (Dandekar *et al.*, 1991).

In vitro sperm capacition accomplished by adding special chemicals to culture medium and incubated under certain conditions for at least one hour to give a chance to make spermatozoa ready to fertilize matured oocyte, those spermatozoa will be successfully undergo acrosomal reaction (AR). In addition, when they are represented in the fertilization culture medium near by the matured oocytes (Moor *et al.,* 1998). Fertilization medium need high amount of protein to fulfill the need of newly form embryo and to support embryonic development must be transferred to another culture medium contains more protein source, ions and growth factors for further development and to get ready for a new environment before embryo transfer (ET) into the female uterus (Parkening and Chang, 1976). The best embryos are the embryos look nice and round and are dividing at a good rate.

Reactive oxygen species (ROS) decrease sperm-oocyte penetration and block sperm-egg fusion in mice (Mammoto *et al.,* 1996). However, binding of sperm to the zona pellucida is promoted by low levels of ROS which they were inhibited by antioxidants (Schroeder *et al*., 1992).

CHAPTE THREE

Materials and

Methods

3.1. METERIALS:

3.1.1. Table 1: Tools and Equipments:

TOOLS / EQUIPMENTS	ORIGIN
Micropipettes	John Poulten Ltd., Great Britain
Petri-dishes , Pasture – Pipettes and syringes	China MEHECO pharmaceuticals and chemicals Imp.& Exp. Corp., China
4-well-Petri dishes	Falcon, 3802, Decton Dickinson lab ware, NJ, USA
Millipore (0.22 and 0.45)µm	Merk, USA
CO_2 incubator	Leec , limited, Private-Nottingham, England.
Dissecting microscope	Will DM3, Switzerland
Dissecting tools & fine dissecting tools	Bunchi , Germany.
Light microscope	Lomo, Russia
Inverted microscope	leitz, wrestler, Germany.
pH-meter	Denki Kagaku Keiki (DKK) ,Japan
Electronic balance	A and D company, Limited, Tokyo, Japan
Oven	Gallenkamp, England
Autoclave	Dixons surgical Ltd. Japan
Laminer air flow	Gelair-Italy
Spectronic 20	Bausch and Lamb , England
Centrifuge	Gallenkamp,England
Refrigerator	National, Japan.
Microtome	Bausch and Lamb , England
Water bath	A and D company, Limited, Tokyo, Japan

3.1.2. Table 2: Chemicals:

NAME	ORIGIN	CONTENTS
Earle's salts medium	Sigma-Aldrich, Uk	Earle's salt (0.884 gm);Na-bicarbonate (0.5 ml); Na-pyruvate(0.001 g); Ampicillin or Penicillin G (0.008 g);deionized distilled water (100 ml);Bovine serum albumin (15 %);pH(7.3-7.4)
RPMI 1640	Sigma-Aldrich, UK	1letter distilled water RPMI 1640 medium base(10.4 gm) Glucose (2.5gm) Hepes (5.986 gm) $Cacl_2$ (0.1 gm) Sodium Pyrouvate (0.1gm) Sodium bicarbonate (2 gm) Penicillin (0.006 gm) Streptomycin (0.0013 gm) Amphotricin (3 ml)
Cholesterol kit reagents	bioMerieux sa 69280 Marcy-l'Etoile/France bioMerieux vitek,Inc.595 Anglum Drive Hazelwood, MO 63042-2395/USA	1.Reagent buffer 1 [Phosphate buffer (0.1 mol/l);Phenol (15 mmol/l);Sodium cholate surfactant (3.74 mmol/l)] 2.Reagent buffer 2 [4-aminoantipyrine (0.5 mmol/l);peroxidase (≥1000U/l);Cholesterol oxidase (≥200 U/l);Cholesterol esterase(≥150 U/l)]

Vitamin A Capsules fish oil 500mg	Biogal pharmaceuticals works ltd	Active compound: addible oil 18:500 mg
Vitamin E	ASIA Pharmaceuticals industries ,Aleppo, Syria	α-tocopheryl acetate 400IU
Sunflower oil	Madrid, Spain, Comity international Geneva	
human postmenopausal gonadotropin (hMG)	Pergonal 500, Serono , Italy	75 IU FSH 75 IU LH
Human chorionic gonadotrophin (hCG)	Profasi, Serono, Italy	5000 IU hCG
Hyaluronidase	(Medi-Cult ,Denmark)	80 IU Hyaluronidase
Bouin's solution	BDH, England.	
Ethanol alcohol	Al Mansour Ltd. , Iraq	
Xylene	Biogal Ltd. ,	
Albumine	Sigma, UK	
Erlich heamatoxylene	BDH, England.	
Paraffin wax	Biogal Ltd.,	
Canada-balsma	Fisher	
Heparin	Denmark	

3.1.3. Animals

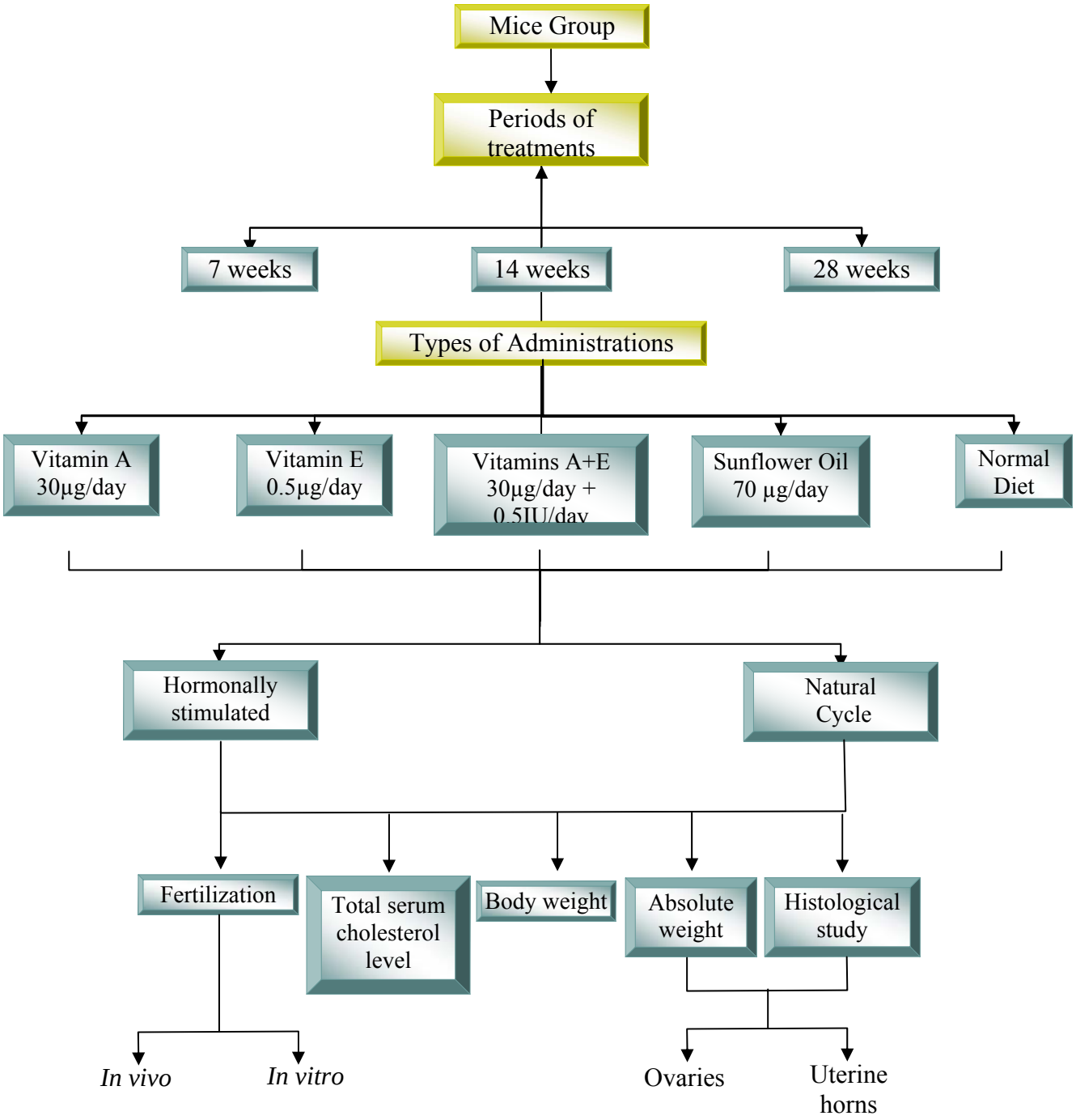
Healthy female and male mice with age (range: 6-8) weeks of weight (19-20) gm of BALB/C strain were obtained from the house of laboratory animals at Biotechnology Research Center. Animals were housed in small plastic cages measured {29x12.5x11.5} cm, each cage contain 6 females. Floors of the cages were covered with the soft crushed wood shaving. The cages were washed once a week with soap and tap water and then sterilized with 70 % ethyl alcohol throughout the period of the study (Peter and Pearson 1961). Animals were kept under suitable environmental conditions such as the temperature of the room which was maintained at range (24-26) º C exposed to 14-hour day light program daily. Tap water using *ad labitum* and pellet were accessible freely (Vidopich and moore, 1992).

3.2. Methods:

3.2.1. Experimental Design:

Female mice were classified into three major groups according to periods of treatment. Also, each major group was subdivided into five minor groups according to type of the antioxidants which were administered orally not exceeded the RDA for each antioxidants: vitamin A, Vitamin E, vitamin A+E and sunflower oil (positive control group). Experimental design was applied in the present study as presented in figure (6).

Figure 6: Experimental design



3.2.2. IVF procedure

3.2.2.1. Superovulation program (SOP):

Female mice were divided into two groups involving natural ovulation group and hormonally stimulated ovulation group. The SOP involved two main steps:

1. First intraperitonial injection (IP) of hMG (10 IU).
2. Secondly (IP) of HCG (10 IU) after 48 hour after the 1st injection (Osborn, 1993).

3.2.2.2 Oocyte collection and preparation:

Oocytes collection and preparation involves a number of steps as fallowing:

1. Female mouse was killed by cervical dislocation after (16-18) hour of HCG injection
2. The reproductive tract was obtained from the abdomen, then washed with sterile normal saline as well as, the entire adipose tissue around it was removed.
3. The tract was placed in a Petri-dish contains protein free RPMI-1640.
4. The oviducts were carefully separated from both ovaries and the uterine horns.
5. Each oviduct was flushed with 1 ml disposable syringe using Earl's culture medium to release all the ovulated oocytes from it. Then, harvested oocytes were collected and washed two times using the same medium. Oocytes treated with hyaluranidase to remove cumulus cells.
6. Oocyte classified into immature, mature and atretic oocytes according to the presence of the first polar body and other morphological features.

7. Immature oocytes incubated for (4-6) hours at 37 º C within 5% CO_2 incubator for *in vitro* maturation. While the mature oocytes incubated with active spermatozoa within 5% CO_2 at 37 º C (Fakhrildin *et al.*, 2001).

3.2.2.3. Sperm preparation and IVF technique:

Sperm preparation, the last step of preparations for *in vitro* fertilization involves the fallowing steps:

1. Spermatozoa were flushed from both vas deferens of mouse with 1 ml of sperm preparation medium, and sperm function tests were examined after incubation for at least 1 hour. Active vassal spermatozoa were adjusted to 1 million /0.5 ml and placed within IVF Petri-dish.
2. Each 5-6 mature oocytes were introduce inside vassal spermatozoa- containing medium
3. Incubated at 37°C in 5% CO_2 incubator for 18-20 hour to assess the Percentages of fertilization and abnormal embryonic development (Fakhrildin *et al.*, 2001).

3.2.3. Measurement of cholesterol level:

The Procedure for measurement of cholesterol level involving a number of steps as fallowing:

1. The working solution was prepared by the contents were reconstituted of one vial of reagent 2 with the contents of one vial of reagent 1, using an adaptor.
2. The solution was mixed by inverting and stores it in the reagent 1 bottle.

3. For standard value 10 µl + 1 ml reagent, Samples: Serum or plasma collected in heparin 10 µl and 1 ml reagent and for zero adjustment reagent blank were used.
4. Wave length of spectronic - 20 were adjusted up to 500 nm (492-550).
5. All the prepared samples, blank and standared were measured after incubation at : 37°C for 5 minutes or 20°C for 10 minutes the color intensity is stable(30 min).
6. Calculations were taken as :(A sample/A standard) × n [(mmol/l:n=5.17);(mg/100ml:n=200);(g/l:n=2)] bioMerieux sa (69280 Marcy-l'Etoile/France bioMerieux vitek,Inc.595 Anglum Drive Hazelwood, MO 63042- 2395/USA).

3.2.4. Histological sections:

After dissection, the organ (uterine horns and ovaries) from female mouse, were weight and fixed in Bouin's solution for 24 hour, and then laid in 70% ethanol and dehydrated by series of ethanol alcohol (70%, 80%, 90%, 95%, and 100%). Then the organs were cleared by xylene then filtered and embedded in paraffin wax (melting point 60°C) in an oven for (4-6) hour. The sample was blocked in paraffin wax, several transverse sections of 5µm thickness were cut using the microtome, and the sections were floated in a water bath at 40 °C. Then (3-5) mounted on slide by using albumin, staining by Eosin and Erlich Haematoxylen, then all were mounted and the cover slip were laid by utilize Canada-balsam. After that sections were examined under light microscope to diagnose the different histological changes; the fallowing parameters were used to evaluate ovarian and uterine horns changes:

1. Ovaries:

1.2. Number and diameter of growing and Graafian follicles.

1.3. Number and diameter of corpora lutea.

2. Uterine horns:

2.1. Epithelial cell layer thickness.

2.2. Diameter of uterine horn (thickness of lining cell+ endometerium+ myometrium).

2.3. Diameter of uterine gland.

In all these histological measurements, ocular and stage micrometers were used (Hameed, 1998).

3.2.5. Statistical analysis

Statistical tests were used depending on the nature of the data. In addition to the standard statistical methods to determine the mean, and standard error of mean, one way analysis (MANOVA) usage program (SPSS) to report the level of statistical significance among the mean of different groups, and use the (T-test) program to determined the significant variations between groups. P-value (0.05) was dependent for the significance level (Soft wear program).

CHAPTER FOUR

Results and

Discussion

4.1. The net body weight increment:

Results of present study pointed that the administration of vitamin E has the best effect on net increment of the body weight throughout different treatment periods of all groups. However, in table (3) non significant (P>0.05) differences in the same parameter were observed between results of administration of vitamin E and groups administered vitamins A+E and positive control (sunflower oil administration) groups throughout treatment periods 14, 28; respectively. It was reported that vitamin E increase and enhance the growth hormones efficiency and interpreted their effects on the net increasing in the body weight regularly (Bardanier *et al.,* 2001). From the results of this study, it was appeared that the administration of mixture of antioxidants, like vitamins A+E, after nearly long-term treatment could cause many benefits for the individual than taken separately. This result in agreement with research was done by (Clarkson, 1995) suggested that antioxidants show synergism positive physiological effects in human being. However long-term of treatment with antioxidants causes an increases in body weight, and these increment in the body weight for groups administered vitamin E and sunflower oil was considered non significant. Stacewicz-Sapuntzakis (1997) reported that the sunflower oil contain more than 20% of vitamin E. therefore, the results of both groups are similar.

Table 3: The net increment in the body weight of mice administrated vitamin A, E and A+E throughout different treatment periods

Group / Treatment periods	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7 weeks	2.7 ± 0.96	5.4±0.54 *	2.2±0.82	1.8±0.68	1.4±0.08
14weeks	11.7±0.94	14.5±1.12 #	13.4±1.31	7.6±1.36	1.2±0.42
28weeks	6.6±1.32	9.5±1.47 †	8.1±1.85	10.4±2.16	6.1±0.33

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05, Weight (grams) (g).*: significant difference. #: significant difference with all groups but vitamin A+E group, †: significant difference with all groups but positive group.

4.2. Body weight of pregnant mice:

Administration of vitamin A, vitamin E and vitamins A+E for 14 weeks have the significant (P<0.05) differences in the body weight of pregnant mice as compared to the other two periods of treatment (Table 4). In addition to increment in the body weight of pregnant mice as a result of number and growth of fetuses with the increased weight of pregnant tissue, however, it was certified that the antioxidants enhance the fertility and anabolism in the female (Palace and Signal, 2001).

From the same table, all groups of pregnant mice administered different types of antioxidants throughout periods of treatment have significant (P<0.05) differences in the body weight when compared to negative control group. These results indicate that the administration of antioxidants for long-term have significant effects on fertility and health of pregnant mice.

Table 4: The body weights for pregnant mice administrated vitamins A, E and A+E before gestation and parturition.

Group / Weeks	Vitamin A		Vitamin E		Vitamins A+E		Positive control		negative control	
	Before gest.	Before part.	Before gest.	Before part.	Before gest.	Before part.	Before gest.	Before part.	Before gest.	Before part.
7 weeks	15.2 ±0.96	21.7 ±1.32	26.02 ±0.54	31.83 ±0.23	21.33 ±0.82	27.1 * ±1.71	14.03 ±0.68	16.85 ±0.26	11.79 ±0.32	14.65 ±0.62
14 weeks	22.42 ±0.94	29.1 ±1.52	25.33 ±1.12	35.5 ±1.49	25.12 ±1.31	37.5 * ±1.49	19.75 ±1.36	22.21 ±0.01	13.14 ±0.92	16.4 ±0.01
28 weeks	28.05 ±1.32	30.66 ±3.38	27.28 ±1.47	30.75 ±3.42	27.6 ±1.85	31.1 * ±3.01	23.21 ±2.16	25.5 ±1.49	17.99 ±2.09	18.02 ±4.19

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05, Weight (grams) (g).* = significant difference with control groups.

Enhancement by vitamins and antioxidant caused by maintaining sex hormones regulation and in some cases these antioxidants replace hormone therapy in aged women (Jill and Manzoni, 2001)

4.3. Litter size:

In general, administration of various antioxidants involving vitamin A, vitamin E, vitamins A+E and sunflower oil for different periods of treatment improved results of litter size as compared to negative control group. However, the best results for litter size were observed when mice administrated vitamins A+E is used as compared to the other groups. Non significant (P>0.05) differences in the litter size were assessed between administration of vitamin E and vitamins A+E groups as compared to the other 28-weeks treated groups (Table 5).

Table 5: The litter size for natural cycle mice administered vitamins A, E and A+E throughout different treatment periods.

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	Negative control
7	7.02 ±1.15	10.5 ±0.49	11.2 * ±0.01	6.33 ±0.33	6.03 ±0.22
14	6.66 ±0.33	7.33 ±0.33	7.6 ±0.24	6.16 ±0.31	6.24 ±0.19
28	3.25 ±0.47	3.75 ±0.25	4.25 * ±0.25	2.11 ±0.29	—

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05, Weight: grams (g).*: significant differences with negative control.

It was known that an increased in the litter size resulted from high regulation of hormonal balance, good general health and high rates of anabolism. Therefore, administration of antioxidants may be enhances and regulates these activities. These results were in agreements with conclusion of research done by James (1998). Moreover, Farwer and co-workers (1994) remarked an enhancement in the number and health of newborn rat babies. However, in this study, using vitamin E with sunflower oil it shows semi-identical results. In addition, the negative control group of mice has no result in litter size (Table 5).

Apparently, it was not recommended to use mice for reproduction when age over 7-9 months as when multiple system disturbances occurred (Peter, 1971). However, a study reveal that giving multivitamin supplementations are a great effects on reproductive tract and sex-hormones regulation were appeared in aged women (Czeizel *et al.*, 1996).

From the results of table 4 and 5, the administrations of antioxidants have synchronized enhancement effects on fertility of females as proved previously by Palace and Signal (2001).

4.4 IVF Program results:

After 7 weeks period of administration of antioxidants, the outcomes of SOP involving vitamin A, vitamin E and vitamins A+E showed non significant (P>0.05) differences in the mean number of ovulated oocytes. However, best percentages for matured oocytes and IVF were noticed for group of vitamins A+E administered-mice. Non-significant (P>0.05) differences were recorded for percentages of abnormal embryonic development of treated and control groups (Table 6-1). The same results were assessed for treated and control groups of mice after 14 weeks period (Table 6-2). Result of mean number of ovulated oocytes, percentage of matured oocytes and percentage of IVF for mice administered vitamins A+E for 28 weeks higher in the percentage than the results of other treated and control groups (Table 6-3). From the same table, non-significant (P>0.05) differences were assessed for percentage of abnormal embryonic development.

Similar results reported by (Czeizel *et al.*, 1996) in his study using multivitamins for aged women and it show a positive effects with reproductive system. In addition, use of antioxidants *in vitro* within culture media improved the outcomes of IVM and IVF for bovine oocytes (Iwata *et al.*, 1998). Furthermore, Halliwell (1996) suggested that administration of a mixture of antioxidants have positive effects on fertility better than the separately taken for each antioxidants. These results are similar to results of administration of vitamins A+E obtained from our study

Table 6: The percentages of *in vitro* maturation, *in vitro* fertilization and abnormal embryonic development from super ovulated (SOP) mice administered vitamins A, E and A+E throughout different treatment periods.

6-1. Group 7 weeks:

Group		mean no. of oocytes/female	Matured oocytes (%)	Fertilized oocytes (%)	Abnormal embryonic development (%)
G1	**Vitamin A**	16	68.75	45.45	25.54
G2	**Vitamin E**	18	73.33	60.33	18.66
G3	**Vitamins A+E**	19 †	74.21 *	68.75 †	20.25
G4	**Positive control**	16	47.5	45.40	25.45
G5	**Negative control**	16	50	55.20	20 .01

†: significant different with all groups but vitamin E, *: significant difference with controlled groups.

6-2. Group14 weeks:

Group		mean no. of oocytes/female	Matured oocytes (%)	Fertilized oocytes (%)	Abnormal embryonic development (%)
G1	**Vitamin A**	15	41.17	38.56	20
G2	**Vitamin E**	17	68.02	58.66	17
G3	**Vitamins A+E**	20 *	70.30 †	66.37 †	18
G4	**Positive control**	13	48.46	43.50	20
G5	**Negative control**	12	46.66	45.33	25

†: significant different with all groups but vitamin E, *: significant difference with controlled groups.

6-3. Group 28 weeks:

Group		mean no. of oocytes/female	Matured oocytes (%)	Fertilized oocytes (%)	Abnormal embryonic development (%)
G1	**Vitamin A**	14	30.57	35	32
G2	**Vitamin E**	16 *	32.15	39	25
G3	**Vitamins A+E**	15	40.34 *	47 #	30
G4	**Positive control**	10	20	36	35
G5	**Negative control**	6	25	35	35

*: significant difference with controlled groups, #: significant different with all groups.

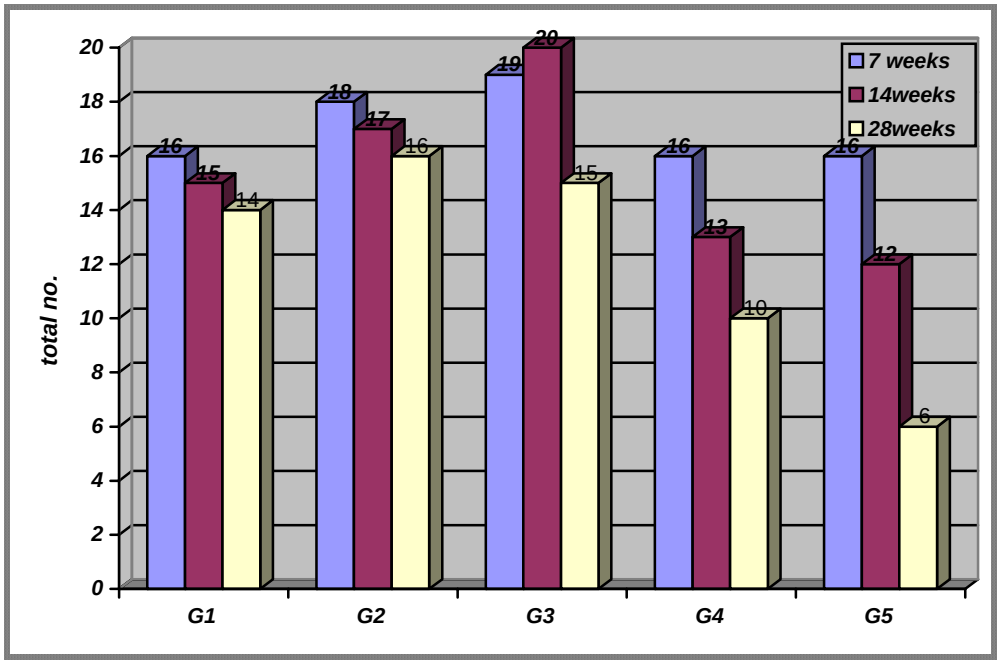


Figure 7-1: comparison for the total number of oocyte collected from treated mice, G1, G2, G3, G4 and G5 represent vitamin A, vitamin E, vitamins A+E, positive control and negative control respectively.

There is non significant (P>0.05) differences between administration of vitamin E alone and group treated with the mixture of the two fat-soluble antioxidant vitamins A+E. In another words, vitamin E give the most effects and combine with vitamin A become more effective. It was reported that vitamin A increases progesterone level in pregnant woman, in turn; an increase thickness of endometrial of uterine horns was noticed and enhancing of the female reproductive system performance (Panth *et al.*, 1991; Palace and Signal, 2001). However, it enhances *in vivo* fertilization outcome more than in the *in vitro* fertilization and that was compatable with the results presented in the tables (6-1, 6-2 and 6-3). Iwata and his colleagues (1998) reported that antioxidants enhance the egg-sperm fusion as a result of scavenge all the ROS that blocks fusion and fertilization.

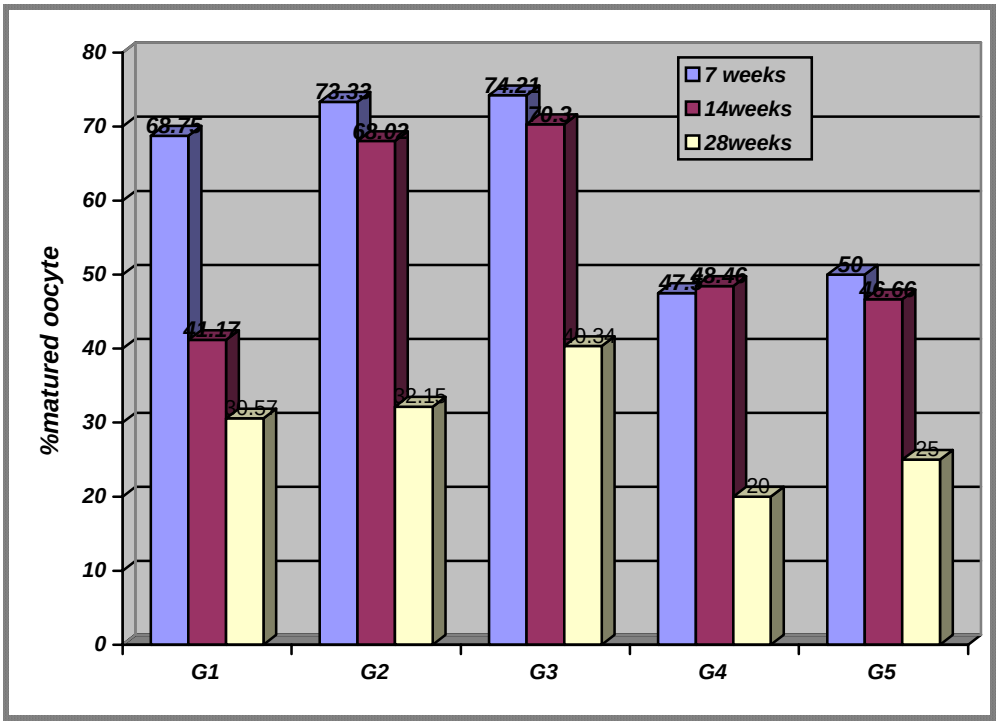


Figure 7-2: comparison for percentage of matured oocyte from the total oocyte collected from treated mice , G1, G2, G3, G4 and G5 represent vitamin A, vitamin E, vitamins A+E, positive control and negative control respectively.

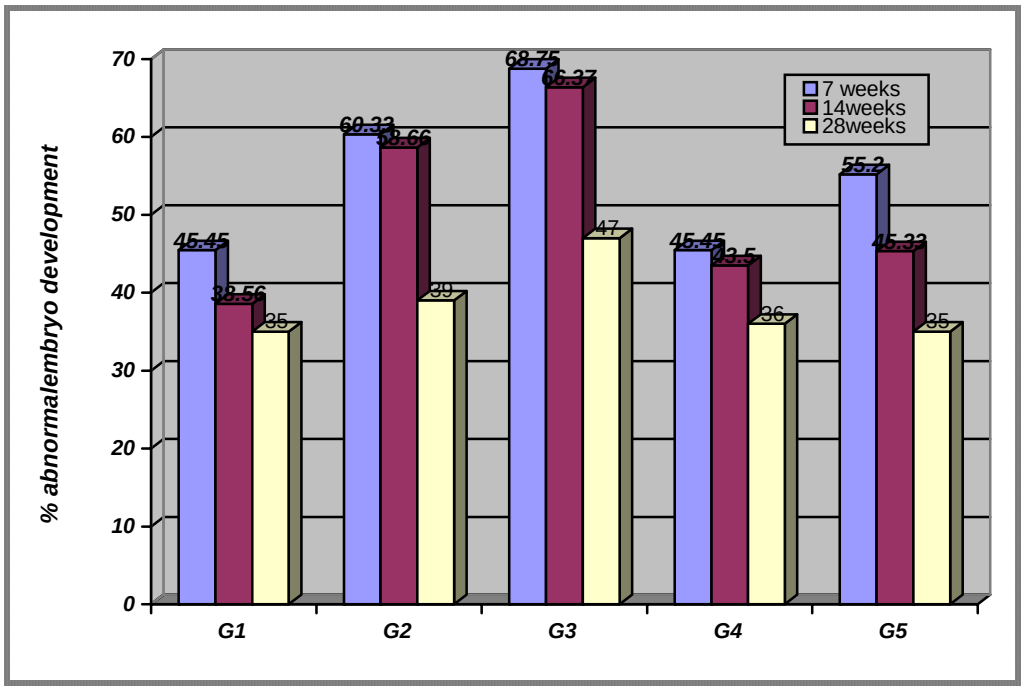


Figure 7-3: Comparison for the percentage fertilized oocyte from the matured oocyte from treated mice. G1, G2, G3, G4 and G5 represent vitamin A, vitamin E, vitamins A+E, positive control and negative control respectively.

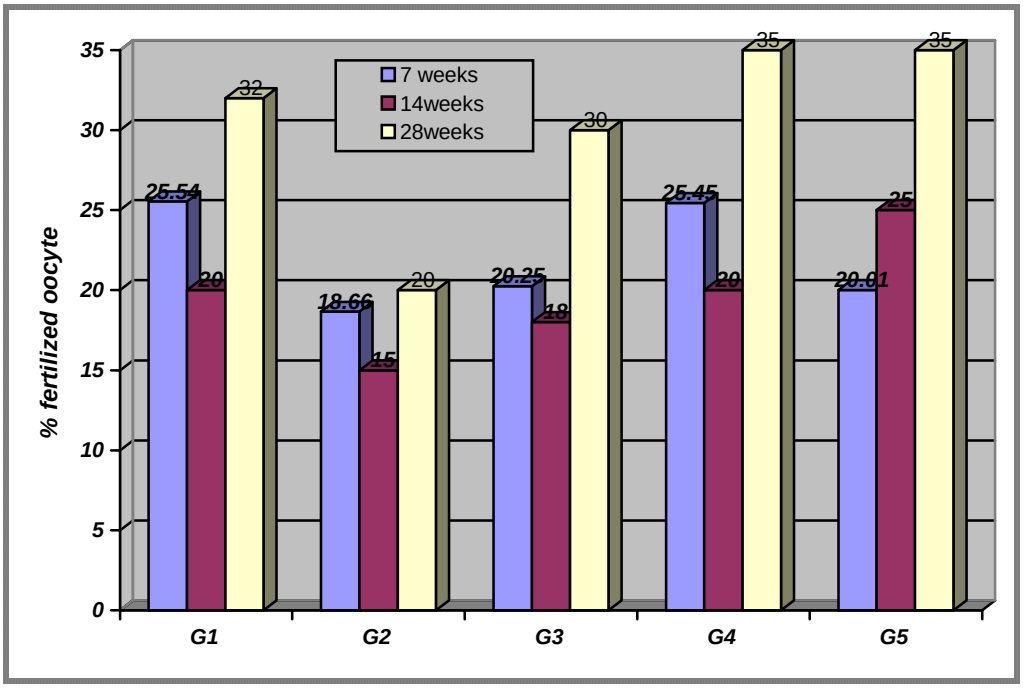


Figure 7-4: comparison for the percentage abnormal development from the fertilized oocyte from treated mice. G1, G2, G3, G4 and G5 represent vitamin A, vitamin E, vitamins A+E, positive control and negative control respectively.

Administration of various antioxidants used in this study for different periods appeared with non significant (P>0.05) differences in the mean number of oocytes. However, the lowest value for mean number of oocytes was recorded for negative control group (Figure 7-1). The flushed mouse oocyte surrounded by cumulus cells was shown in (Figure 8). These results pointed to positive effects of these fat-soluble antioxidant vitamins on follicular growth and ovulation process, and these effects through regulation of effects on sex hormones. However, these two effects continue post-ovulation.

Results of the present study indicated that the administration of vitamin A, vitamin E, and vitamins A+E for 7 and 14 weeks periods have the best percentages for matured oocytes as compared to 28 weeks period of vitamins administration (Figure 7-2). It was known that the requirements for IVM are the constituents of culture medium microenvironment for IVM and competence of immature

oocytes to be matured *in vitro* (Wirtham and Witmyer, 1988). Therefore, adding those antioxidants to culture medium have positive effects on regulating enzymatic activities deals with IVF (Thatcher and Decherney, 1989) and those antioxidants scavenging the ROS which enhance the percentage of IVM (Choi *et al.*, 1987; Dandekar *et al.*, 1991). matured oocytes showed in (Figure 9).

Figure (7-3) shows the percentages of IVF for mice administered antioxidants for 7, 14 and 28 weeks periods. It was noticed that the administration of vitamins A+E have the best results for percentage of IVF. However, non-significant ($P>0.05$) differences in the percentage of IVF were reported between groups of mice administered vitamin E and vitamins A+E. These results of present study certified the positive effects of antioxidants on IVF as appeared for results of IVM. Figures (10 and 11) they show the normal development of embryo. Similar results for IVM / IVF were obtained in the study by Iwata and co-workers (1998).

Significant ($P<0.05$) differences in the percentages of abnormal embryonic development were assessed for groups of mice administrated various antioxidants for 14 weeks period as compared to 7 and 28 weeks treatment periods (Figure 7-4). Abnormal embryo shown in figure (12) Lowest percentage of

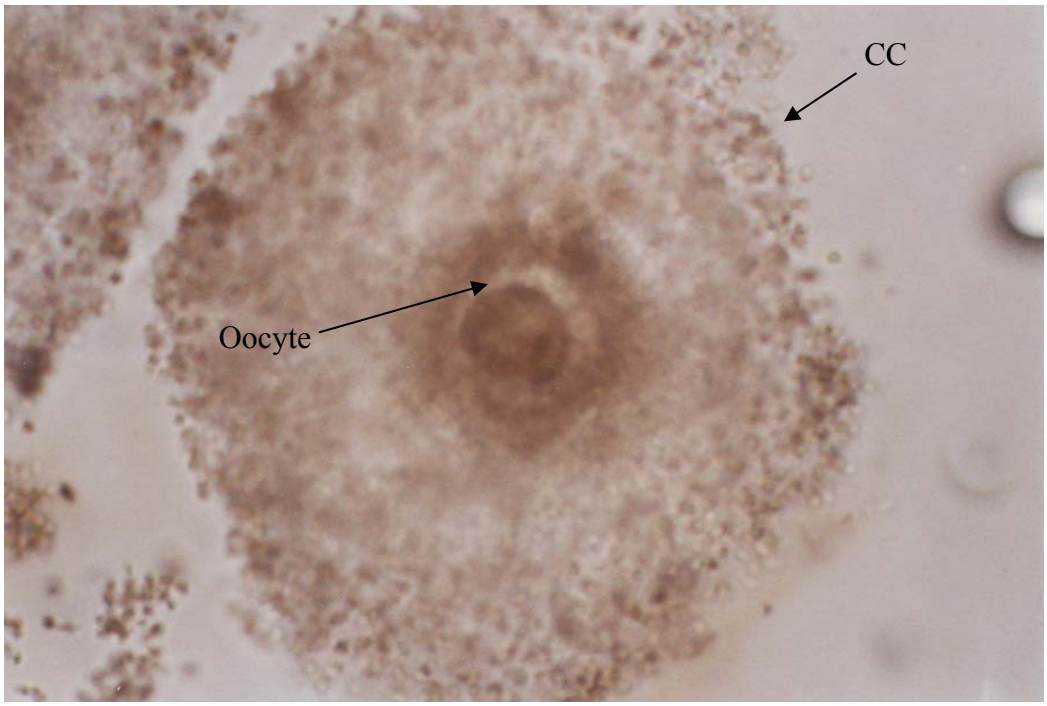


Figure 8: Flushed mouse oocyte surrounded by cumulus cells (CC) under magnification power (180X)

abnormal embryonic development was obtained from mice administered vitamin E for 14 weeks as compared to the other treated and control groups.

These antioxidants not only enhance the ovulation process and IVM/IVF outcomes but continuous action of these antioxidants appeared through reduction of the abnormal embryonic development. Several studies reported the impacts of ROS on progressive embryonic development and causes multiple disturbances of metabolism and cleavage of blastomeres (Osborn, 1993; Volarcik, *et al.,* 1998). Therefore, typical effect of antioxidant through scavenger of ROS, then abnormal embryonic development was reduced well. Moor, *et al.* (1998) considered the development of abnormal embryos as one and important factor for reduction of embryo transfer.

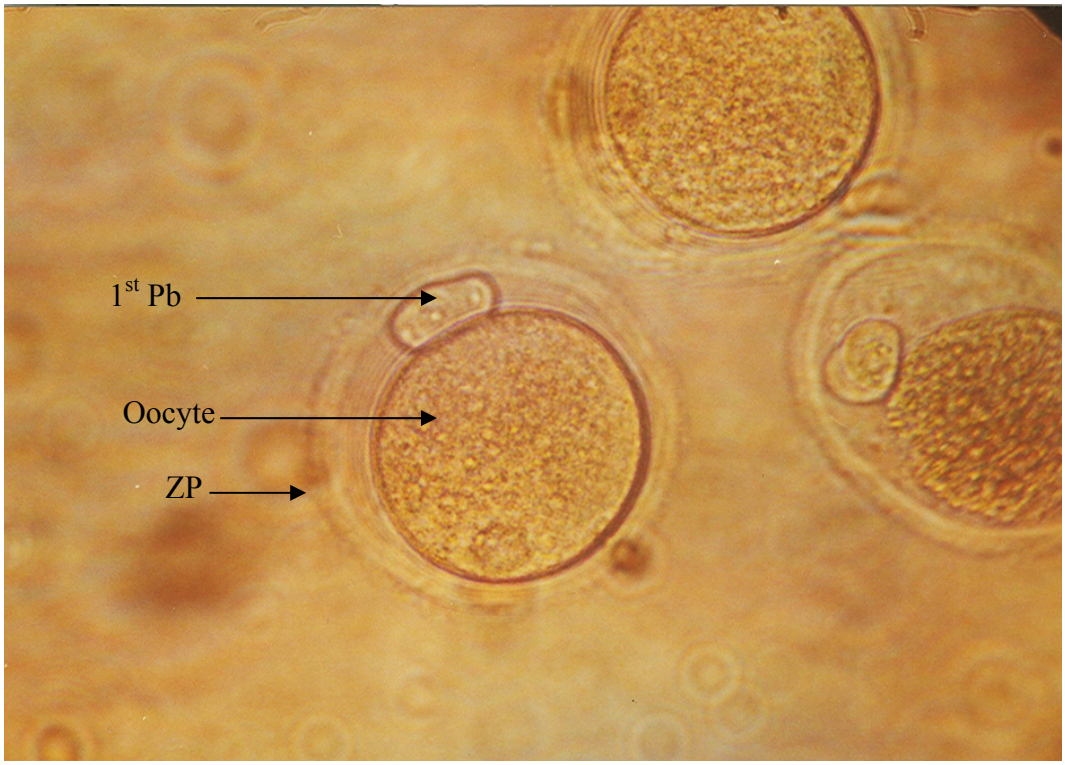


Figure 9: Matured mouse oocyte and 1st. polar body (1st Pb), surrounded by Zona pellucida (ZP) under magnification power (400X)

From the same figure, non significant (P>0.05) differences were observed among treated and control groups of mice administered for 28 weeks period. These results confirm that using vitamins enhances the reproductive system performance and the fertilization process (Crary and McCarty, 1984). For the aged female, changes in the reproductive tract could cause poor fertility (Nava *et al.*, 1991; Fitzgerald *et al.*, 1994).

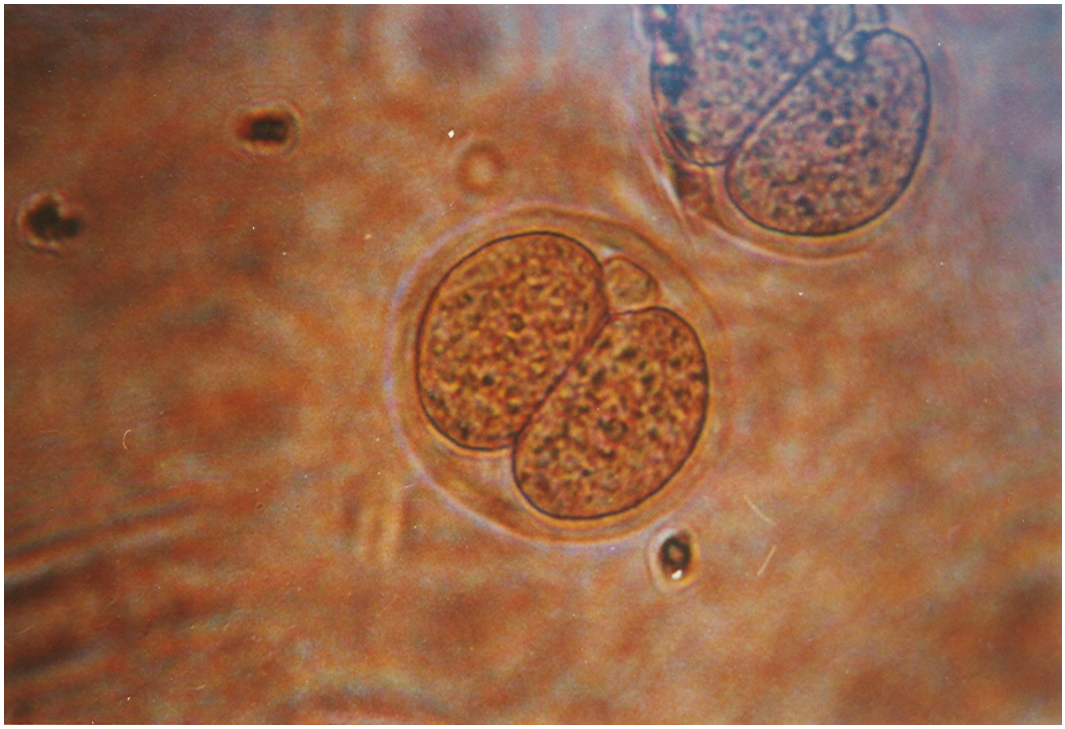


Figure 10: Two cells mouse embryo. Magnification under power (400X)

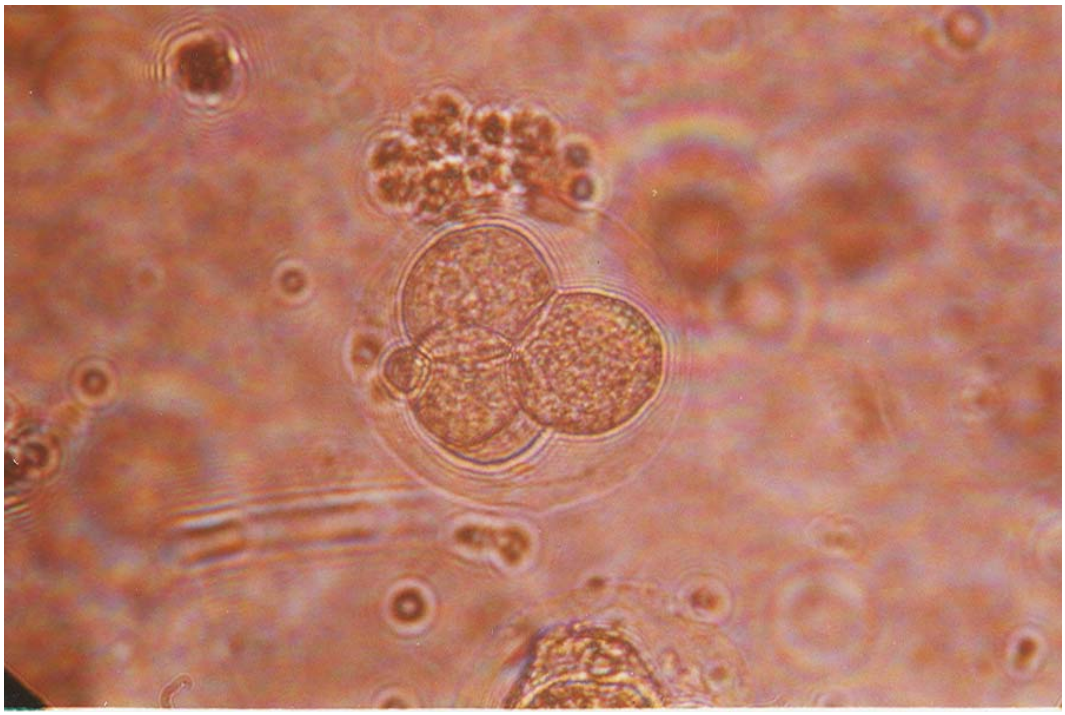


Figure 11: four cells mouse embryo. Magnification under power (400X)

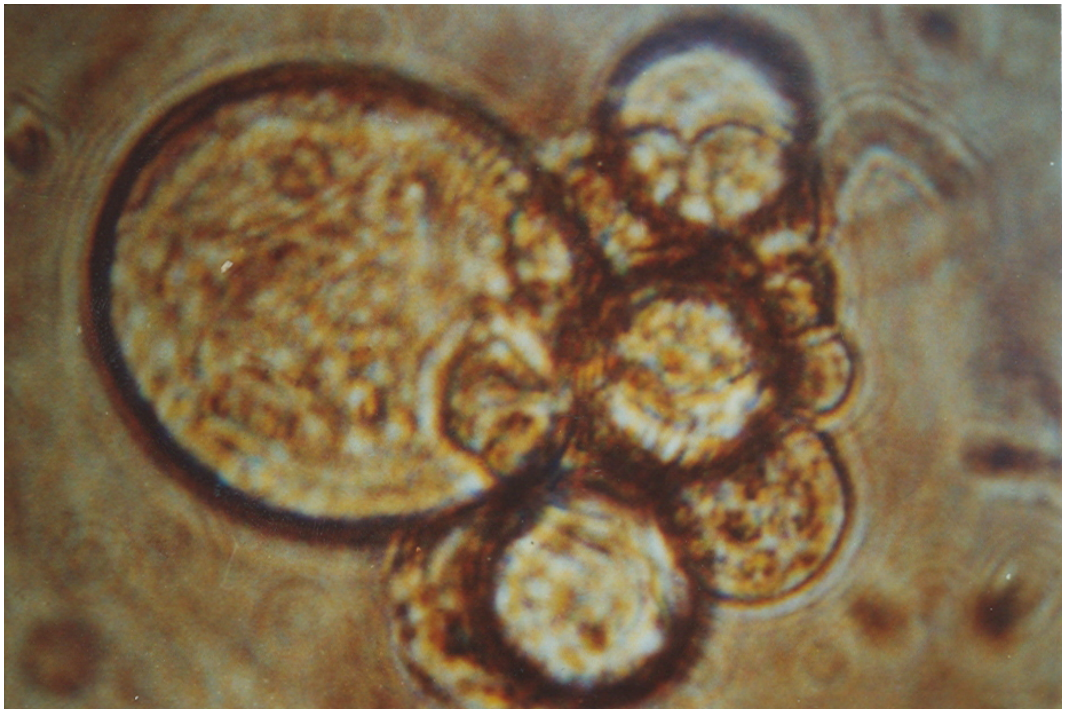


Figure 12: Abnormal mouse embryonic development. Magnification under power (1600X)

4.5. Absolute ovarian weight:

Table 7-1 shows the effect of administration of different antioxidants used in this experiment for the three treated periods on absolute ovarian weight (AOW) in mice with natural cycle. It was observed that the administration of vitamin A has the best and significant ($P<0.05$) effect on AOW as compared to the other groups treated for 7 and 14 weeks period. Vitamin A has direct effects on sex hormones related to enhance the ovarian performance (Bendich and langseth, 1989). It was mentioned that the increment number of follicles and its size lead to increase the AOW (Benson and Talaro, 1996). Moreover, increased number of ovarian follicles coupled with highly active ovaries (Biggers *et al.*, 1962). Significant ($P<0.05$) differences were reported between group of hormonally stimulated-mice administrated vitamins A+E for 7 weeks period when compared to other treated and control

groups. However, it was noticed that the administration of vitamins A+E for 14 weeks causes significant ($P<0.05$) differences as compared to positive and negative control groups (Table 7-2). Non-significant ($P>0.05$) differences were assessed among all 28 weeks treated and control groups of mice hormonally stimulated (Table 7-2).

Hormones, gonadotropins, their induction of multiple follicular growth leads to ovulation because of ovarian cortex activity (Dukelow, *et al.,* 1983). Therefore, an increased in AOW is under the effect of antioxidant administration, which causes an increase in follicular growth and number. Studies reported that vitamin E and vitamin A enhances performance of reproductive organs and secretion of sex hormones respectively (Green, 1972; Ford ad Sowell, 1999).

Table 7: The absolute ovarian weight for mice administered vitamins A, E and A+E throughout different treatment periods.

7-1: Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	0.09 ±0.02 #	0.05 ±0.09	0.06 ±0.02	0.03 ±0.01	0.03 ±0.01
14	0.08 ±0.01 #	0.02 ±0.04	0.05 ±0.03	0.04 0.02	0.04 ±0.03
28	0.07 ±0.04 #	0.03 ±0.03	0.05 ±0.01	0.04 ±0.01	0.03 ±0.01

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05, Weight: grams (g), #: significant difference all groups.

7-2: Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	0.13 ±0.02	0.12 ±0.09	0.19 # 0.03	0.11 ±0.02	0.11 ±0.02
14	0.15 ±0.02	0.14 ±0.03	0.18 * ±0.06	0.12 ±0.02	0.11 ±0.05
28	0.12 ±0.04	0.11 0.03	0.14 ±0.02	0.11 ±0.04	0.10 ±0.03

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05, Weight: grams (g).*: significant difference with negative control group, #: significant difference all groups.

After 7 weeks period of administration of various antioxidants, it was shown that the results of AOW for groups of hormonally stimulated-mice were better in comparison with the results of AOW for natural cycle-mice groups as presented in the figure (13-1). Same results were reported for 14 and 28 periods of treatment (Figure 13-2 and 13-3). From these results, it was suggested that the antioxidants administered to mice may stimulate better regulation for gonadotropins action and synergistic with action of ovarian tissue receptors. However, the results of present study are in agreement with conclusion of research conducted by Guthrie (1995)

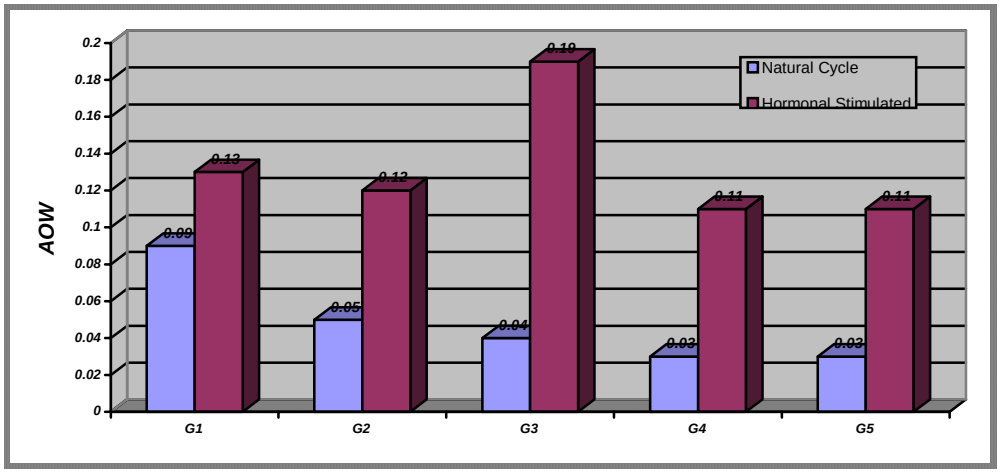


Figure 13-1: after 7 week's treatment.

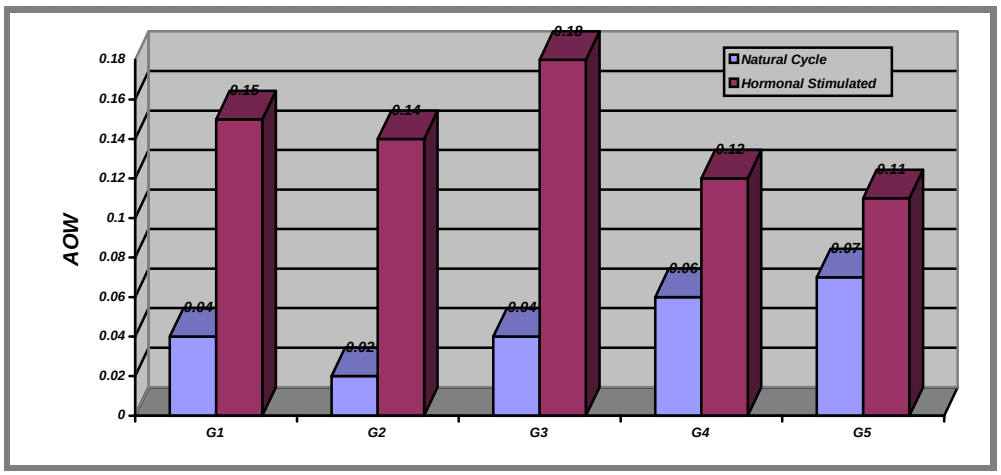


Figure 13-2: after 14 week's treatment.

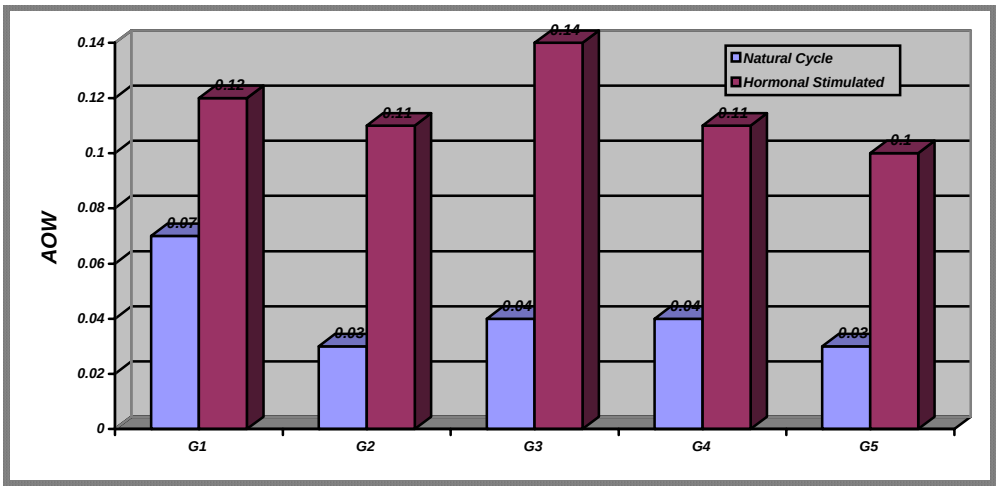


Figure 13-3: after 28 week's treatment.

Figure 13: Comparison between absolute ovarian weight for natural cycle- and hormonally stimulated-mice administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

4.6. Absolute uterine weight:

Depending on absolute uterine horns weight (AUHW) results of the present study it was appeared that the groups of natural cycle-mice administered vitamin A and vitamins A+E for 7 weeks period have the best results for AUHW as compared to the other treated and control groups. However, best results for AUHW were obtained with natural cycle-mice administrated vitamin E and vitamins A+E for 14 and 28 weeks periods as compared to the other groups (Table 8-1). It was certified that vitamin A administration support most tissue build of body system including reproductive system and uterine performance especially (Crary and McCarty, 1984). However, this effect was propagated after long-term administration of mixture antioxidants as appeared from results of this study. In addition, these results are similar to those obtained by Engstrom, (2001). However, an increased in weight of uterine horns refers to the endometrium and myometrium thickness, and finally refers to enhancement in performance (Halliwell and Gulteridge, 1989).

In general, the administration of the antioxidants for the three periods of treatment to hormonally stimulated-mice have results for AUHW better than the results of negative control group (Table 8-2). Meanwhile, non-significant ($P>0.05$) differences for AUHW were assessed between positive and negative control groups (Table 8-2). According to figures (14-1, 14-2 and 14-3), different treatment periods involving 7, 14 and 28 weeks of administration of these different antioxidants have the results of AUHW for all groups of hormonally stimulated-mice which are better than those of all groups of natural cycle-mice.

Table 8: The absolute mice uterine weight administered vitamins A, E and A+E throughout different treatment periods.

8-1. Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	0.18 ±0.01	0.12 ±0.01	0.19 ±0.02	0.09 # ±0.01	0.12 ±0.01
14	0.09 ±0.01	0.15 ±0.04	0.16 * ±0.03	0.09 ±0.03	0.11 ±024
28	0.08 ±0.01	0.13 ±0.01	0.13 * ±0.01	0.08 ±0.01	0.03 ±2.32

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05

Weight: grams (g).*: significant difference with all groups but vitamin E, #: significant difference all groups.

8-2. Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	0.20 * ±0.02	0.18 ±0.02	0.19 ±0.01	0.15 ±0.01	0.13 ±0.01
14	0.19 # ±0.01	0.19 ±0.02	0.20 ±0.01	0.14 ±0.01	0.13 ±0.34
28	0.19 # ±0.01	0.18 ±0.03	0.20 ±0.01	0.08 ±0.01	0.07 ±3.21

Number of mice for each group (6), Value=Mean± SEM, significance level: P <0.05

Weight: grams (g).*: significant difference than negative control, #: significant difference than control groups.

All of these results may explain the cooperative effect of antioxidants and hormonal stimulation on increased weight through tissue building of reproductive system. An increased amount of progesterone secretion after ovulation (Gosden, 1975) vitamin A increases the production of prostaglandin, which enhances blood supplementation to uterine tissue and increasing the uterine tissue (Gonen *et al.,* 1989; Eugstrom, 2001). Increase in prostaglandin during the luteal phase leads to increases in the thickness of endometrium and myometrium preparing it to possible gestation (Farwer *et al.,* 1994).

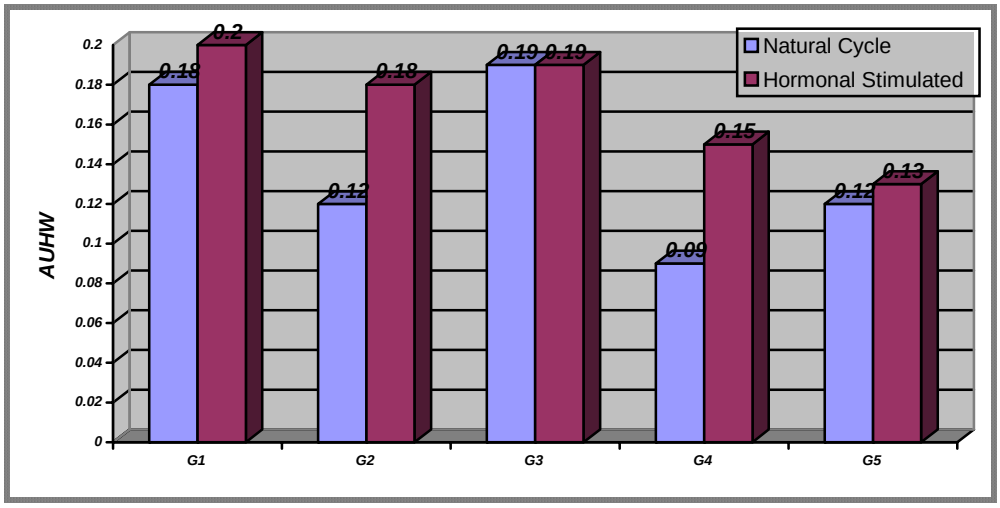


Figure 14-1: after 7 weeks of treatment.

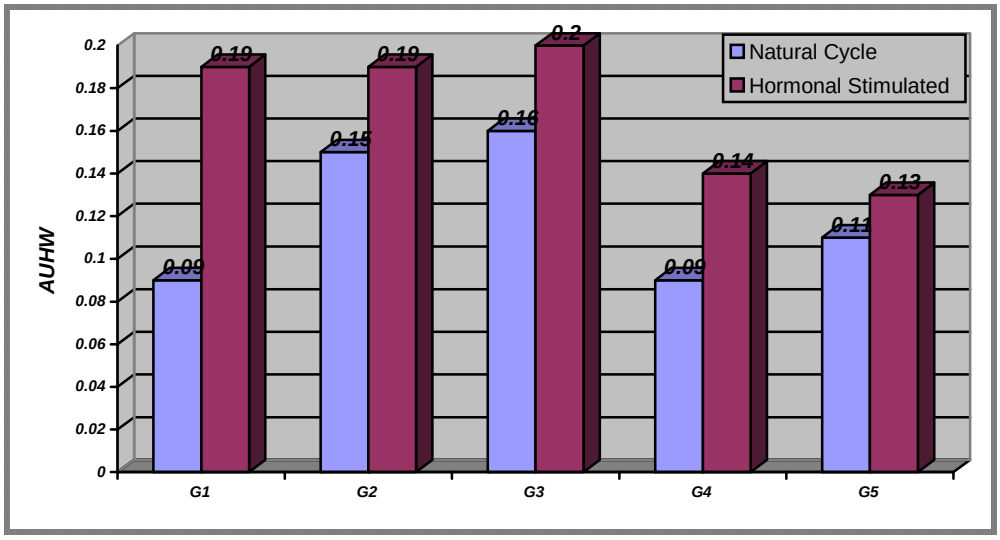


Figure 14-2: after 14 weeks of treatment.

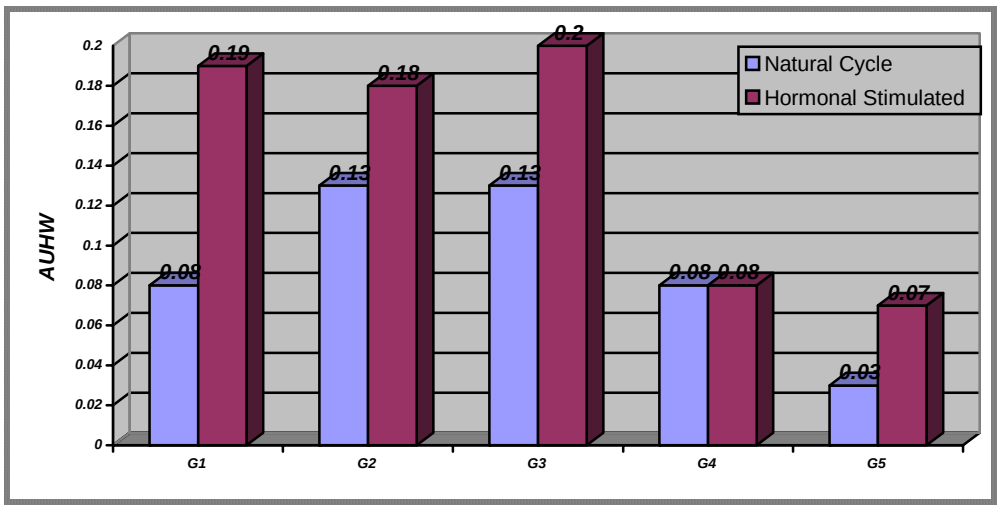


Figure 14-3: after 28 weeks of treatment.

Figure 14: Comparison between absolute uterine horn weight for natural cycle and hormonally stimulated mice. Administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5; respectively.

4.7. Uterine horn longitudinal sections study:

4.7.1. Epithelial cell layer thickness:

The administration of these various antioxidants used for 7, 14 and 28 weeks increased thickness of epithelial cell layer of endometrium as compared to negative control group of natural cycle-mice (Table 9-1). It was observed that vitamin E has the best results when compared to groups administered vitamin A and vitamins A+E (Table 9-1). Generally, vitamin E enhances the formation and maintenance of the epithelial layers all over the body (Gougeon *et al.,* 1994). Increased thickness of epithelial cells layer means that activity of these cells are high. In addition, ovarian progesterone increases activity of these cells(Dukelow and Ridha, 1988).

As was reported, the sex hormones stimulated in response to administration of antioxidants and vitamin E especially (Chan, 1993). After 7, 14 and 28 weeks of antioxidants administration, thickness of epithelial cell lining uterine horn of hormonally stimulated-mice administered vitamin E was significantly ($P<0.05$) increased when compared to the control groups (Table 9-2).

Many factors affect the reproductive system after vitamins administration, which collectively enhances performance of reproduction through regulation of sex hormones, ROS scavengers, and enhance tissue anabolism (Azen *et al.,* 1996; Anderson and Young, 2003). Further effect also produced through hormonal stimulation of ovarian follicular growth, which in turn appeared as more progesterone secretion (Gonen *et al.,* 1989). When uterine tissue becomes ready for gestation their gives a sign for the most and certain action of progesterone (Ishikawa and Endo, 1996).

Gradual reduction was observed in the thickness of epithelial cell layer in the treated and control groups of mice throughout different periods of administration (Table 9-2). It is known as getting older, the thickness of epithelial cell layer lining uterine horn reduced, in addition to physiological changes (Kardinnal *et al.,* 1994). Treated groups show less changes in that layer regards to vitamins ability to maintain this layer.

Figures (15-1, 15-2 and 15-3) show results of thickness of epithelial cell layer lining uterine horn of mice administered various antioxidants for 7, 14 and 28 weeks periods. It was reported that the hormonally stimulated-mice have results better than the natural cycle-mice. Normally, hormonal treatment of mice of induction the multiple follicular growth and ovulation this effect stimulation for more progesterone secretion of corpus luteum (Hodgen, 1989). Therefore, epithelial cells lining uterine horn increases its activity under progesterone effect.

Some of progesterone and epithelia cells activity was regulated by antioxidants (Jacob and Burri, 1996). Synchronized effects of hormonal stimulation and antioxidants clearly appear on the hyperfunction of these epithelial cells and followed by increased thickness (Salonen *et al.,* 1985), this layer was well defined in (Figure 17).

Table 9: Epithelial cell layer thickness of mice administered vitamins A, E and A+E throughout different treatment periods.

9-1. Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	22.13 ±2.88	25.92 * ±2.86	20.32 ±0.01	19.9 ±3.51	14.92 ±2.33
14	20.35 ±4.99	21.74 † ±0.14	19.31 ±2.86	15.54 ±2.23	12.7 ±2.23
28	17.01 ±0.57	19.03 † ±0.57	17.50 ±0.01	12.52 ±0.16#	11.2 ±2.11

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), Significant level: P<0.05, *: significant differences with negative group, †: significant differences with control groups.

9-2. Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	Negative control
7	25.51 ±2.88	26.8 † ±0.16	22.53 ±0.01	18.52 ±2.76	15.25 ±0.81
14	21.42 ±0.14	26.25 † ±4.99	20.32 ±0.14	15.92 ±0.45	15.25 ±0.22
28	17.52 ±0.57	20.52 * ±0.01	18.32 ±0.16	14.53 ±2.86	12.64 ±2.52

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), Significant level: P<0.05, *: significant differences with negative group, †: significant differences with control groups.

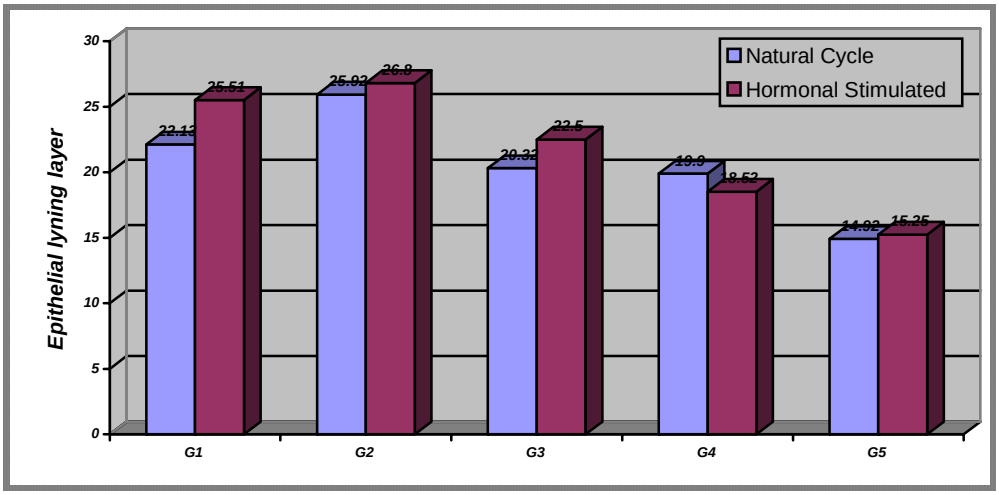


Figure 15-1: 7 weeks of treatment.

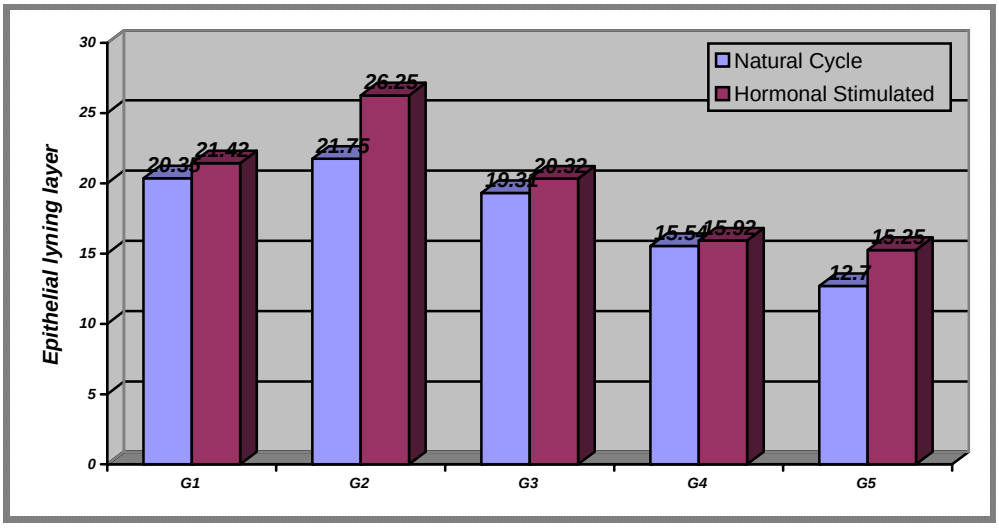


Figure 15-2: 14 weeks of treatment.

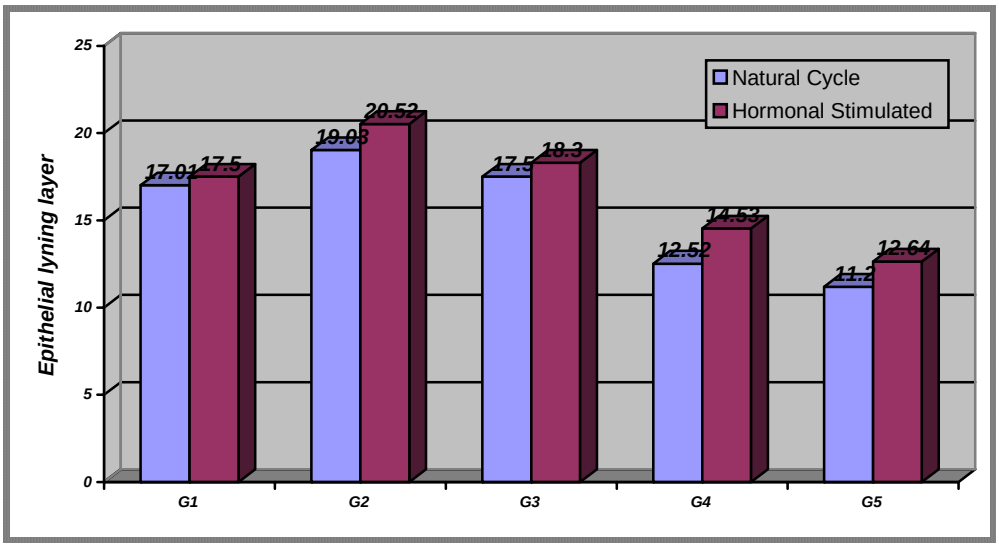


Figure 15-3: 28 weeks of treatment.

Figure 15: Comparison between natural cycle- and hormonally stimulated-mice in epithelial cell layer thickness administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5; respectively.

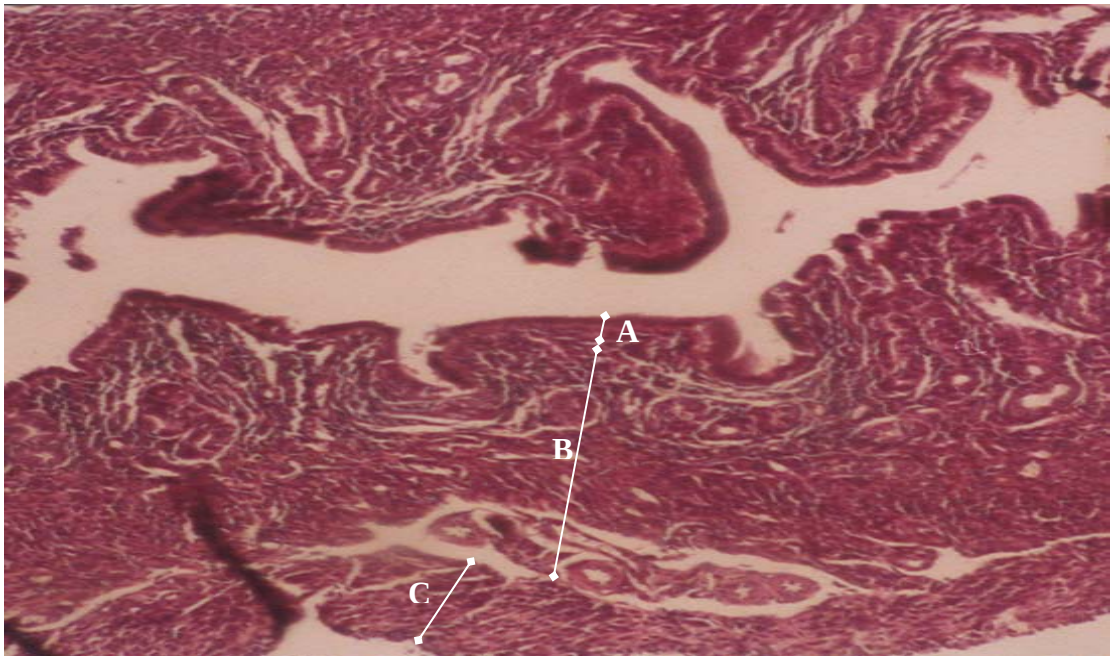


Figure 16: Longitudinal section of uterine horn for naturally cycle-mice group negative control treated for 7 weeks, stained by Heamatoxylen and Eosin dyes, under magnification power(40X), A:(Epithelail lining, B:(Endometrium), C:(Myomaterium)
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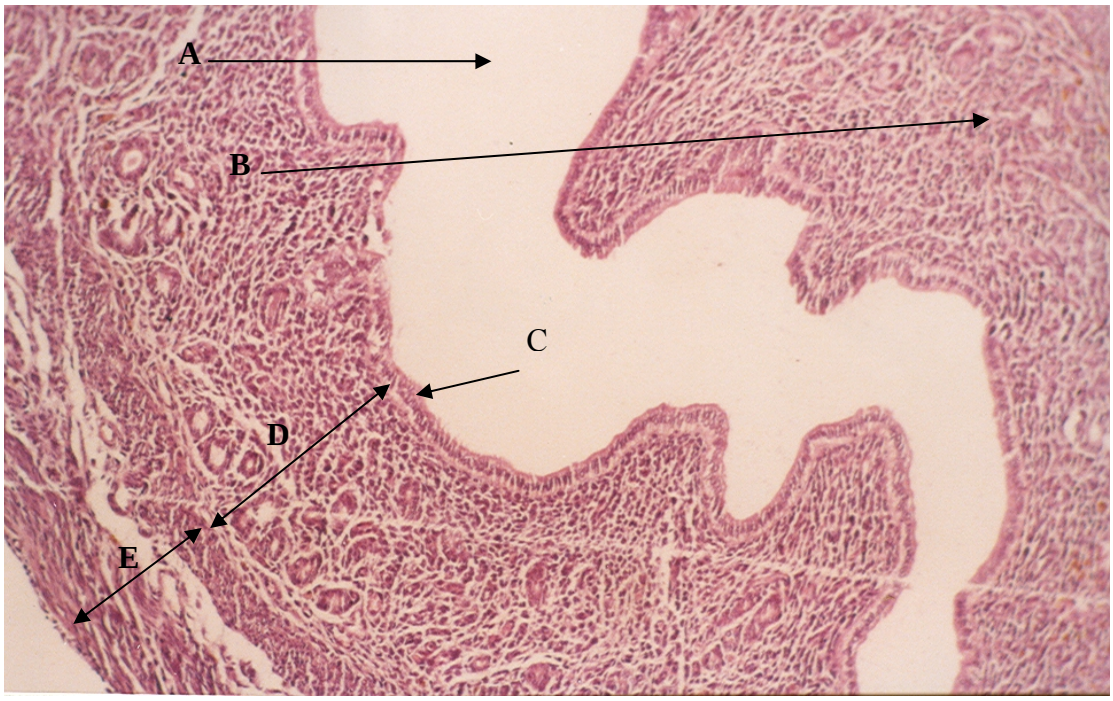

Figure 17: Longitudinal section in uterine horn for 7 weeks treatment with vitamin E for hormonally stimulated-mice. A :(Uterine horn lumen), B :(Uterine Gland), C :(Epithelial lining cell layer), D :(Endometrium), E: (Myomaterium), C+D+E: (Diameter of uterine horn), stained by Heamatoxylen and Eosin dyes, under magnification power (40X).

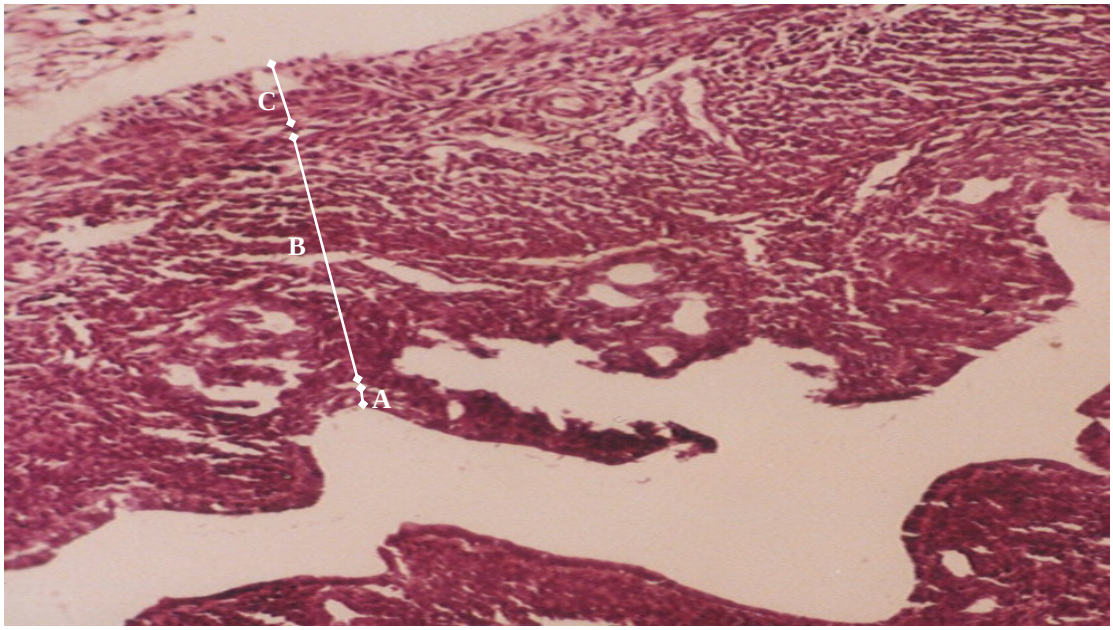

Figure 18: Longitudinal section in uterine horn for mice after 28 weeks Positive control naturally cycle, stained by Heamatoxylen and Eosin dyes, under magnification power (40X). A:(Epithelail lining, B:(Endometrium), C:(Myomaterium)

4.7.2. Diameter of uterine horn:

After 7 and 14 weeks of antioxidants administration, vitamin E has the best and significant ($P<0.05$) increase in the diameter of uterine horn for natural cycle- and hormonally stimulated-mice as compared to other treated and control groups (Tables 10-1 and 10-2). As it is shown in figure (17), endometrium and myometrium well-defined layers have great thickness when compared to same layers in the figure (16), which it represents the negative control after 7 weeks treatment of naturally cycle-mice. While non-significant ($P>0.05$) differences in the diameter of uterine horn were assessed among treated and control groups of natural cycle-mice after 28 weeks period of administration of various antioxidants (Table 10-1).

Table 10: Diameter of uterine horn of mice administered vitamins A, E and A+E throughout different treatment periods.

10-1: Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	740.67 ±1.75	1010.35 # ±1.45	400.25 ±2.12	390.65 ±0.65	370.04 ±2.09
14	580.52 ±8.5	840.66 * ±0.82	670.53 ±2.08	280.31 ±0.09	260.76 ±2.39
28	270.35 ±0.57	300 ±0.41	300.32 † ±1.15	250.79 ±0.57	240.39 ±0.31

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with control groups, #: significant difference with all groups, †: significant differences with control groups.

10.2. Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	840.67 ±1.66	1050 # ±1.15	710.5 ±1.49	520.04 ±0.31	400 ±0.02
14	850.33 ±1.21	940.67 ±0.99	690.52 * ±0.99	310.65 ±0.01	310.77 ±0.04
28	350.65 ±1.15	580.33 † ±1.33	500.53 ±0.57	300 ±1.15	280.35 ±0.76

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with control groups, #: significant difference with all groups, †: significant differences with control groups.

From these results, administration of antioxidants has results better than the negative control group. In this study, using fat soluble-vitamins as antioxidants involved in many activities, collectively support body fertility and health, especially in age progression (Sies, 1993; Clarkson, 1995). Although, aging causes aging features on reproductive system (Zuccotti *et al.,* 1998), as it shown in (figure 18) positive control treated group in 28 week reveals a histological changes (less thickness and regularity in uterine horn layers) in comparing to (Figure 17) when vitamin E treated group show the most distinguished regular layers of uterine horns .

From natural cycle- and hormonally stimulated-mice, non significant ($P>0.05$) differences were noticed in the diameter of uterine horn between positive and negative control groups of mice throughout 7, 14 and 28 weeks (Tables 10-1 and 10-2). This result was certified by Page (1981) who mentioned that the sunflower oil has no significant positive effects on reproductive system.

Figures (19-1, 19-2 and 19-3) show the results of diameter of uterine horn for natural cycle- and hormonally stimulated-mice administered antioxidants. Non significant ($P>0.05$) differences were reported between groups of natural cycle-mice and groups of hormonally stimulated-mice, except the groups of vitamins A+E and vitamin A administered for 7 and 14 weeks; respectively. However, it was clear that diameter of uterine horn for hormonally stimulated-mice administered vitamin E and vitamins A+E increased significantly ($P<0.05$) as compared to natural cycle-mice (Figure 19-3).

In general, these results refer to efficiency of vitamin E alone or combined with vitamin A in supporting the reproductive system, through increased diameter of uterine horn to be more receptive for embryos especially for aged female (Jill and Manzoni, 2001). However, Tomas and co-workers (1997) reported that the sex hormones therapy for infertile female with endocrine disorders and/or aged has less potential activity for ovarian induction. Moreover, vitamins A support female reproductive system (Palace and Signal, 2001).

Similarly, vitamin E support male and female fertility (Thiessen *et al.,* 1975). Studies suggested that sex hormones have a synergism activity with vitamins (Salonen *et al.,* 1985). All these studies give a good explanation for a question, whey the reproductive systems enhance and maintained its performance with antioxidants in hormonally stimulated-mice more than any other groups?

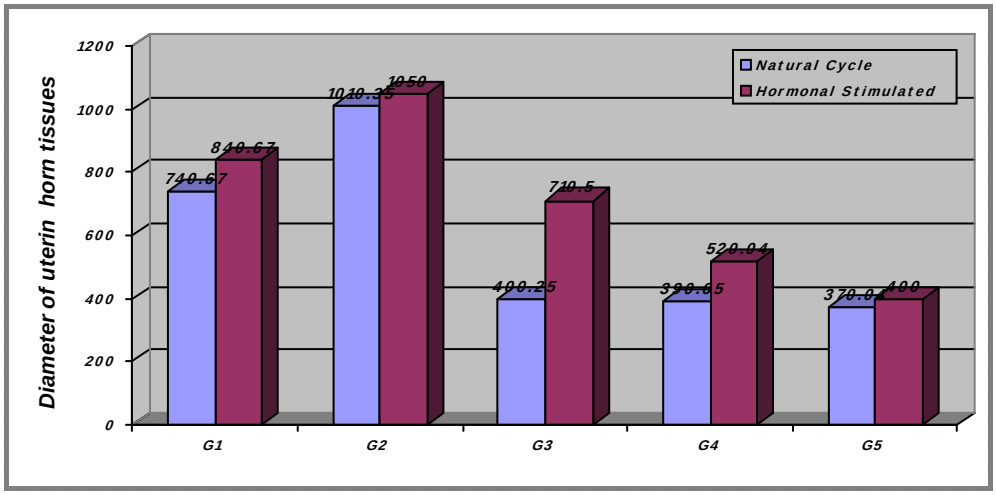

Figure 19-1:7 weeks of treatment.

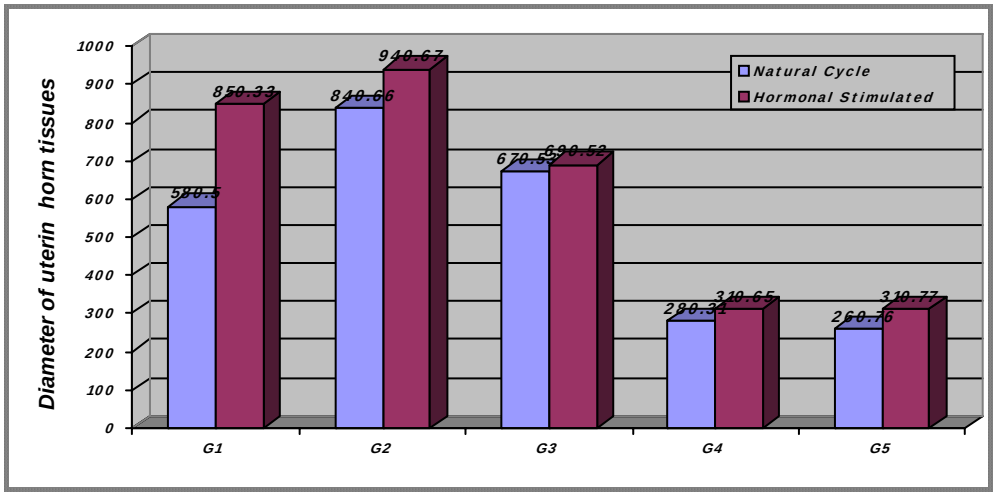

Figure 19-2: 14 weeks of treatment.

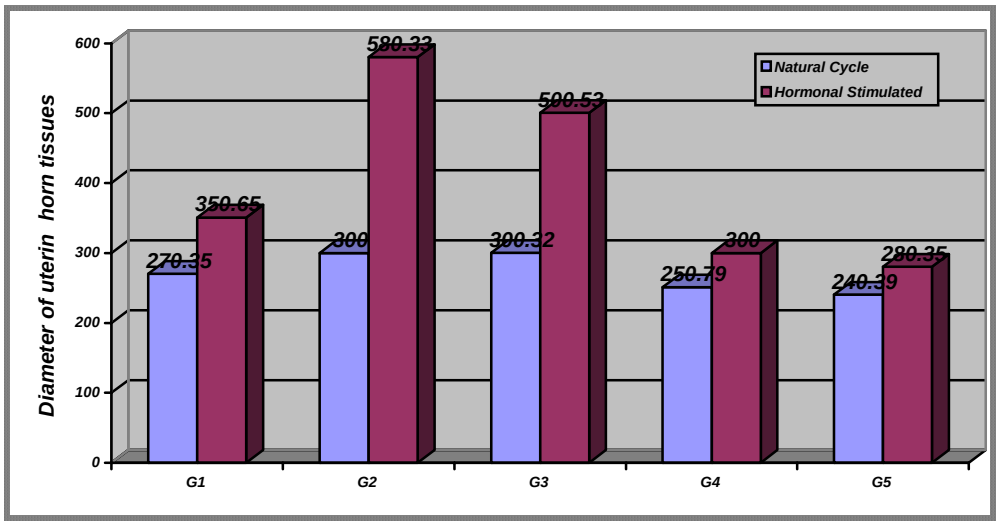

Figure 19-3: 28 weeks of treatment.

Figure 19: Comparison between natural cycle- and hormonally stimulated-mice in the diameter of uterine horn tissues for all groups administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

4.7.3. Diameter of uterine gland:

Non significant (P>0.05) differences were indicated in the diameter of uterine glands of natural cycle-mice after 7 weeks of administration of various antioxidants used in the present study (Table 11-1). From the same table, administration of vitamins A+E for 14 weeks was significantly (P<0.05) increased as compared to other groups of mice administered vitamin A, positive control and negative control, however, non-significant (P>0.05) differences were assessed between groups of mice administered vitamin E and vitamins A+E for 14 weeks. Moreover, best result in the diameter of uterine glands was achieved after administration vitamin E for 7 weeks to hormonally stimulated-Mice (Table 11-1).

Enlargement of uterine glands is under progesterone effect, and the growth of these glands is negatively affected as age increased and/or reduction in the progesterone level (Banett, 1994; Jungueira *et al.,* 1995). The morphological properties, activities and numbers of uterine glands is a reflex to the activity of uterine tissue and function of ovarian hormones (Zurcher *et al.,* 1982). The effect of administration of vitamins as antioxidants may be propagated as the period of administration is continued. Therefore, significant differences were observed after 14 and 28 weeks of administration. It was reported that the long-term treatment has accumulatively effects (Block *et al.*, 2001).

After 14 and 28 weeks of administration of vitamins A+E, best results were obtained as compared to other groups. Mixture of antioxidants shows better effects than taken separately.

Table 11: Uterine gland diameter of mice administered vitamins A, E and A+E throughout different treatment periods.

11.1. Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	positive control	Negative control
7	50.24 ±0.31	62.9 ±0.44	50.51 † ±0.31	40.21 ±2.43	45.32 ±0.09
14	41.35 ±0.41	62.55 ±0.31	75.6 * ±0.39	37.75 ±0.87	35.67 ±0.67
28	34.21 ±0.44	50.54 ±0.63	55.21 † ±0.44	—	—

Number of mice for each group (6), Values: Mean± SEM, diameter: (μm), significant level: P<0.05, *: significant differences with all groups, †: significant differences with control groups.

11.2. Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	positive control	Negative control
7	55.42 ±0.31	87.53 ±0.76	80.31 † ±0.39	50.21 ±0.76	50.23 ±0.81
14	81.55 ±0.67	75.59 ±0.01	81.63 † ±0.41	46.5 ±0.23	50.92 ±1.23
28	65.03 ±0.37	50.31 ±0.31	70.33 † ±0.44	30.04 ±0.39	35.13 ±0.67

Number of mice for each group (6), Values: Mean± SEM, diameter: (μm), significant level: P<0.05, †: significant differences with control groups.

Vitamin E supports the reproductive system and the absorption of vitamin A (Bramley *et al.,* 2000), meanwhile, vitamin A has potential effects on uterine performance (Lithgow and Politzer, 1977). Therefore, these changes in uterine glands may refer to these vitamins activity including vitamins A+E and vitamin E alone for administered 14 weeks. Non significant (P>0.05) differences were assessed among groups of mice administered vitamin A, vitamin E and vitamins A+E for 7, 14 and 28 weeks. However, significant (P<0.05) increased was noticed for groups of mice administered vitamin A, vitamin E and vitamins A+E for 7, 14 and 28 weeks as compared to positive and negative control groups (Table 11-2). Studies reported that antioxidants may enhance the female reproductive system to stimulat hormones (Gozan, 1992; Palae and Signal, 2001).

4.8. Ovarian longitudinal sections study:

4.8.1. Growing follicles number and diameter:

The number of growing follicles (Gr.F) of natural cycle-mice administered vitamin A for 7 and 14 has the best results when compared to other groups (Table 12-1). Vitamin A has potential effects on folliculogensis and ovarian performance (Panth *et al.,* 1991). However, administration of vitamin E for 28 weeks has the best number for growing follicles when compared to other groups (Table 12-1). It was mentioned that vitamin E has an accumulative effects in regarding to the ovarian performance (Azzi *et al.,* 2000). From the same table, the mean diameter of (Gr.F) was significantly (P<0.05) increased after administration sunflower oil and vitamins A+E for 7 and 14 weeks; respectively as compared to groups administered vitamin A and vitamin E. sunflower oil group treated

for 7 weeks. These results may indicate that mice of all groups were within fertility age; however, these results of treatment with vitamins A+E for long-term, were better than that when each vitamin used separately. (Wildman and Medeiros, 2000) mentioned same result, while, best and non significant results may indicate that mice of all groups were within fertility age; however, these results of treatment with vitamins A+E for long-term, were better than that when each vitamin used separately. (Wildman and Medeiros, 2000) mentioned same result, while, best and non significant ($P>0.05$) increase in the diameter of (Gr.F) was seen in mice administered vitamins A+E for 28 weeks when compared to other groups (Table 12-1). Certainly, the ovarian section is a reflex to fertility female, it must contain much number of follicles weather they were growing or Grafian follicles (Zuckerman, 1991). In aged female, the number of both types of follicles is reduced because of the retardation of folliculogenesis process (Zuccotti *et al.,* 1998). However, this conclusion assists our results for groups naturally cycle-mice; with the exception of the group of mice, administered vitamins A+E group after 28 weeks of treatment. Table (12-2) shows the effect of various antioxidants administration on number and diameter of ovarian (Gr.F) of hormonally stimulated-mice. Highest number of Gr.F was achieved when vitamin A and vitamins A+E used for 7 weeks. While, vitamin A and vitamin E have the highest number in Gr.F after 14 weeks of treatment, On the other hand, non significant ($P>0.05$) differences were occurred among treated and control groups after 28 weeks of antioxidant administration. Different studies reported that these antioxidants vitamins improve production of sex hormones and folliculogenesis process by enhancing the ovaries performance (Clarkson, 1995).

Table 12: Number and diameter of growing follicles on ovary of mice administered vitamins A, E and A+E throughout different treatment periods.

12-1: natural cycle group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	Positive control	No.	Negative control
7	6	150.25 ±0.76	3	90.85 ±0.83	5	50.28 ±0.16	3	170.9 ***** ±1.82	2	120.4 ±1.52
14	7 †	70.13 ±0.42	6	92.78 ±0.73	6	250.16 ***** ±1.78	2	170.42 ±0.76	2	150.08 ±0.87
28	4	130.37 ±1.34	6	150.3 ±0.64	6 #	170.91 † ±0.47	2	130.35 ±0.46	2	100.34 ±0.98

Number of mice for each group (6), Values: Mean± SEM, diameter: (μm), significant level: P<0.05, *: significant differences with all groups, #: significant difference with all groups but vitamin E, †: significant differences with negative control group.

12-2: Hormonally stimulated group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	positive control	No.	negative control
7	10	210.78 ±1.21	5	150.04 ±2.33	***** 10	80.32 ±0.76	5	220.09 ±1.22	3	150.3 ±1.82
14	# 12	70.11 ±0.42	12	90.91 ±3.64	9	150.04 ±0.47	3	140.93 ±0.33	4	170.52 ±0.71
28	6	130.32 ±0.42	7 †	180.33 ±1.27	6	140.78 ±0.35	4	160.66 ±0.44	4	120.7 ±0.46

Number of mice for each group (6), Values: Mean± SEM, diameter: (μm), significant level: P<0.05, *: significant differences with all but vitamin A group, #: significant difference with all groups but vitamin E, †: significant differences with control groups.

Higher diameter of ovarian (Gr.F) of hormonally stimulated-mice administered sunflower oil, vitamins A+E and vitamin E after 7, 14 and 28 weeks; respectively (Table 12-2). These results showed increased numbers and diameter of ovarian (Gr.F) hormonally stimulated more than with natural cycle-mice.

That was obvious in figures (20-1, 20-2 and 20-3) show administration of various antioxidants to mice for 7, 14 and 28 weeks was increased number of Gr.F of hormonally stimulated-mice better than the natural cycle-mice.

Normally, ovarian stimulation using gonadotropin injection causes more follicles to be growing (Mahadevan *et al.,* 1985). Furthermore, administration of vitamins as antioxidants offers further enhancement on ovarian folliclogenesis. It was known that the synergism action of antioxidants with hormonal stimulation prepared the germinal layer within ovarian cortex for better folliculogensis (Paker, 1992; Pandy *et al.,* 2000).

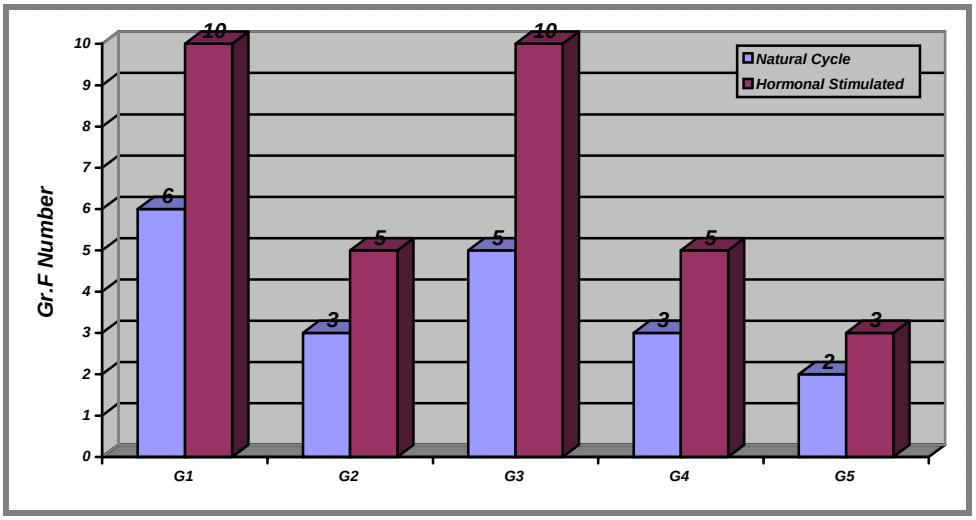

Figure 20-1: 7 weeks of treatment.

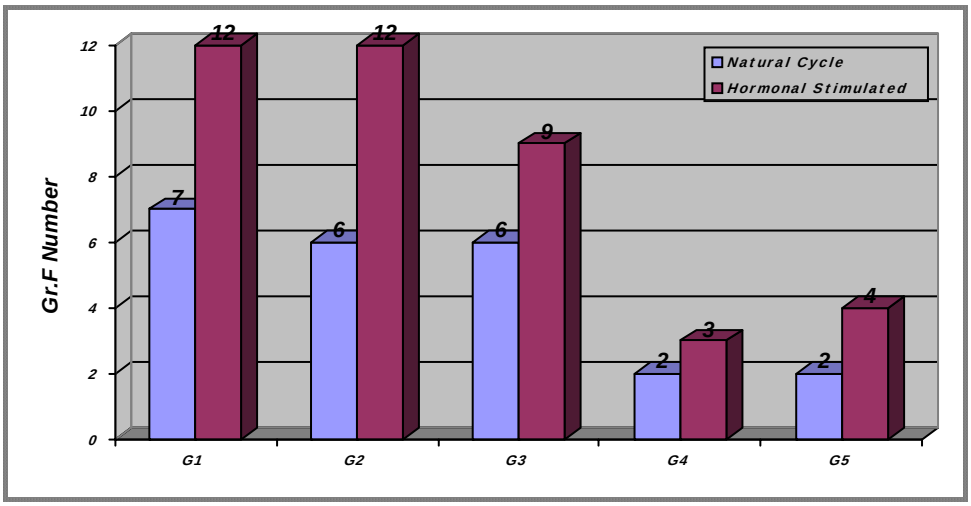

Figure 20-2:14 weeks of treatment.

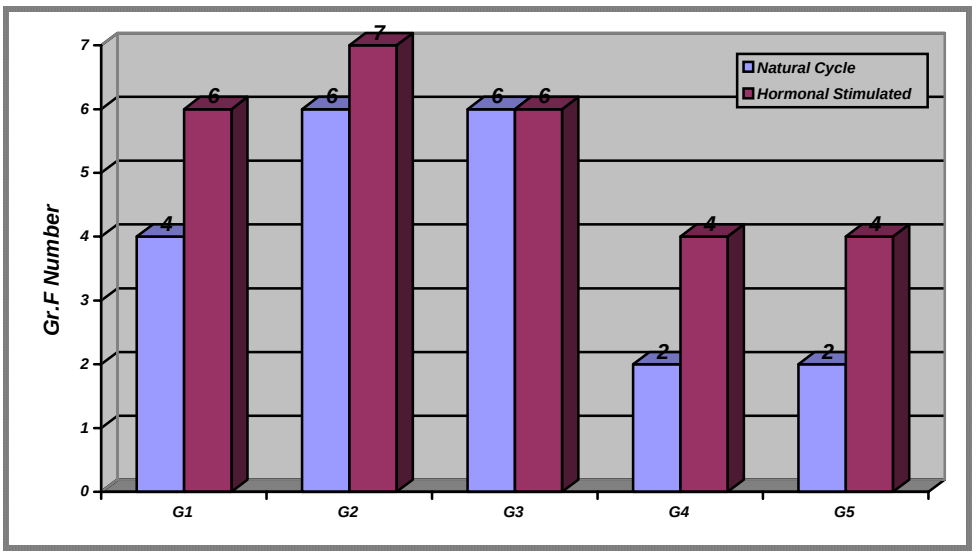

Figure 20-3: 28 weeks of treatment.

Figure 20: Comparison between natural cycle- and hormonally stimulated-mice for number of growing follicles number for all groups administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

4.8.2. Graafian's follicles number and diameter:

Results of the present study appeared that the administration of vitamins A+E for 7, 14 and 28 weeks has the best number of ovarian GF of natural cycle- and hormonally stimulated-mice as compared to treated and control groups (Tables 13-1; 13-2). Non significant (P>0.05) differences were assessed among groups of mice administered vitamin A, vitamin E and vitamins A+E for 7, 14 and 28 weeks. It was observed that treatment with vitamins A and E separately or mixed improves ovarian performance (Salonen *et al.,* 1985). Also, no significant (P>0.05) differences were noticed between positive and negative control groups (Table 13-1). Sunflower oil has non-defined positive effects on fertility (Pryde *et al.,* 1981). Therefore, no significant enhancement in ovarian performance concerning to folliculogenesis as compared to negative control group.

Best results for mean diameter of ovarian G.F. of natural cycle-mice were recorded when vitamin E administrated for 7 and 14 weeks. While, administration of vitamins A+E for 28 weeks has the best results for diameter of G.F. (Table 13-1). These results may certify needs for further antioxidants vitamin with age progression to enhances the performance of ovaries more than the young female mice. An increased in the age is accompanied with impaired some physiological and histological changes, therefore, more vitamins supplementation is referred to repair or maintain performance of the reproductive system (Sies, 1997). Administration of vitamin A to hormonally stimulated-mice for 7, 14 and 28 weeks have the highest diameter of ovarian G.F as compared to other treated and control groups (Table 13-2).

Table 13: Number and diameter of Graafian follicles on ovary of mice administered vitamins A, E and A+E throughout different treatment periods.

13.1. Natural cycle group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	positive control	No.	negative control
7	3	375.52 ±0.28	3	650.24 * ±3.27	4	375.52 ±0.49	2	496.92 ±0.3	2	200.04 ±0.98
14	4	600.5 ±1.49	3	604.49 † ±0.07	5	460.2 ±0.28	2	225.42 ±1.4	2	170.43 ±0.87
28	2	250.24 ±3.27	2	345.9 ±0.49	3	370.4 ±1.31	—	—	—	—

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with all groups, †: significant differences with control groups.

13.2. Hormonally stimulated group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	positive control	No.	negative control
7	4	533.6 * ±1.31	4	450 ±0.57	4	375.5 ±1.49	3	491.83 ±0.99	3	225.5 ±1.05
14	5	725.2 * ±2.5	5	608.17 ±1.21	5	575.5 ±1.61	3	549.98 ±0.3	3	200 ±0.93
28	3	600.4 # ±0.57	2	350.11 ±0.57	4	345.9 ±1.32	—	—	—	—

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with negative group, #: significant difference with all groups

Vitamin A has a potential effect on ovaries, concerning an increasing in the folliculogenesis process and enhance the ovulation process by accelerating the transformation of growing follicles to Graafian follicles (Panth *et al.,* 1991).

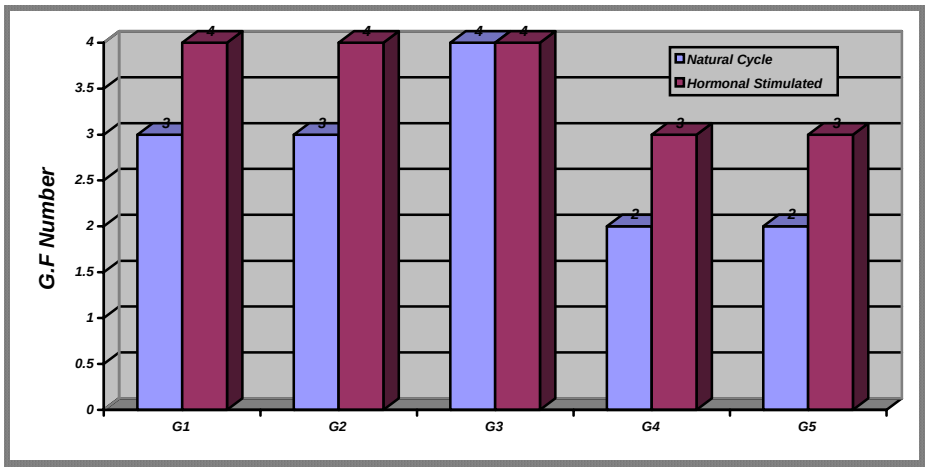

Figure 21-1: 7 weeks of treatment.

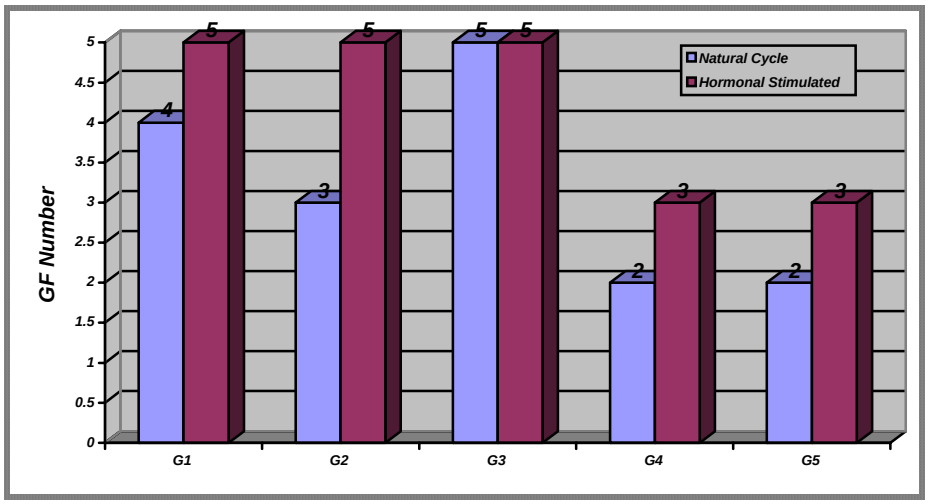

Figure 21-2: 14 weeks treatment.

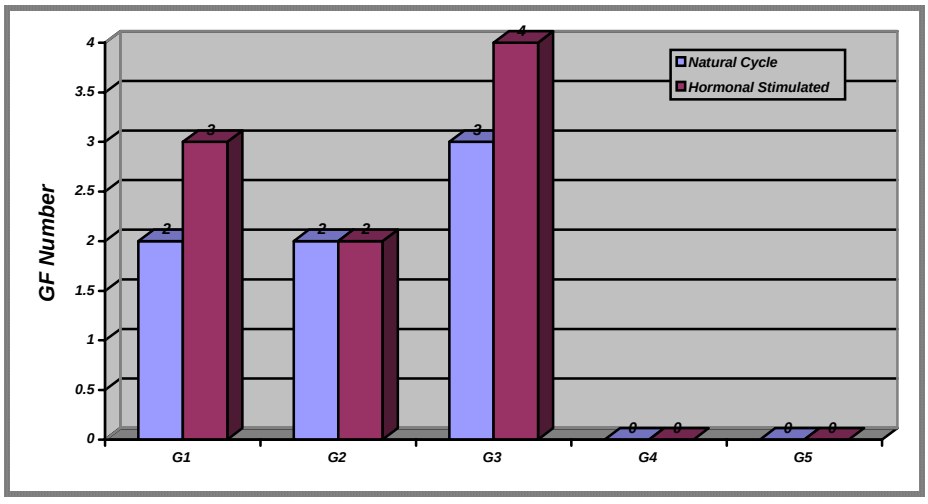

Figure 21-3: 28 weeks treatment.

Figure 21: Comparison between natural cycle- and hormonally stimulated-mice in Graafian follicles number for all groups administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

The effect of antioxidants used in this study for different periods on number of G.F of natural cycle- and hormonally stimulated-mice were presented in figures (21-1, 21-2 and 21-3). Better results in the number of Graafian's follicles were achieved for hormonally stimulated-mice than the natural cycle-mice, however, these do not reach level of significance (P=0.05). That may partially agreed with previous study showed that the synergism activity between sex hormones and antioxidants enhancing fertility achieved (Salonen *et al.,* 1985).

4.8.3. Number and diameter of corpus luteum:

Effects of administration of various antioxidants used in this study for 7, 14 and 28 weeks on number and mean diameter of corpus luteum (CL) were presented in the table (14-1). After 7 and 14 weeks periods of administration, there is a non-significant (P>0.05) difference in the number of CL between treated and control groups of mice. Meanwhile, significant (P>0.05) reduction was observed in the number of CL of negative control group as compared to other groups of mice administered various antioxidants (Table 14-1). Therefore, with post-ovulation, more numbers of CL was obtained. Similar results were noticed by Dawood (1994). Furthermore, number of CL firmly deales with the number of ovulated oocytes from the single ovaries (Browder, 1985). Higher diameter of CL were achieved for the group administrated vitamin A and vitamin E for 7, 14 and 28; respectively as compared to treated and control groups (Table 14-1).

Table 14: Number and diameter of corpus luteum on ovary of mice administered vitamins A, E and A+E throughout different treatment periods.

14-1: Natural cycle group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	positive control	No.	Negative control
7	5	470.91 * ±1.24	5	310.75 ±1.75	6	350.15 ±0.64	3	350.32 ±0.22	4	301.03 ±0.99
14	6	380.33 ±2.77	5	450.25 * ±2.77	6	420.12 ±2.92	4	360.43 ±0.04	5	320.19 ±0.54
28	4	220.03 ±5.24	3	350.12 * ±1.73	4	280.33 ±1.09	2	270.12 ±0.85	2	250.02 ±0.88

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with all groups.

14-2: Hormonally stimulated group

Group / Weeks	No.	Vitamin A	No.	Vitamin E	No.	Vitamins A+E	No.	positive control	No.	negative control
7	6	500.13 ±2.31	7	520.41# ±2.3	8 *	450.84 ±1.69	4	360.72 ±0.1	5	390.83 ±0.22
14	6	560.66 ±2.63	6	430.31 ±1.89	7	460.66 ±3.05	5	350.32 ±0.65	5	370.03 ±0.04
28	5	380.51 ±2.35	4	420.16 † ±4.49	5	370.82 ±1.32	4	310.71 ±2.09	3	350.13 ±0.85

Number of mice for each group (6), Values: Mean± SEM, diameter: (µm), significant level: P<0.05, *: significant differences with all groups, #: significant difference with all groups but vitamin A, †: significant differences with control groups.

(Browder, 1985) reported that the shape and diameter of CL has no true impression on fertility of ovaries inregard to folliculogenesis and ovulation. (Einspanier, 1997) indicated that number and diameter of CL deals nothing with ovarian activity more than it refer to the number of the oocytes that have been ovulated before.

Administration of vitamins A+E for 7 and 14 weeks period has the highest number of CL for hormonally stimulated-mice as compared to treated and control groups. However, after 28 weeks period of antioxidants administration for hormonally stimulated-mice, non-significant ($P>0.05$) differences were indicated in the number of CL between treated and control groups. These results along with the results of the numbers of growing follicles and Graafian follicles revealed a positive correlation. These results may indicate that treatment with antioxidants enhances the performance of ovaries remarkably through increasing numbers of GrF, GF and CL of treated groups. However, the number of CL refers to the number of the just ovulated oocytes (Banett, 1994).

Best results were achieved for diameter of CL of hormonally stimulated-mice after administration of vitamin E and vitamin A for 7 and 14 weeks periods as compared to other groups (Table 14-2). It was very clear that the results of short-term treatments with those vitamins were similar to those treated for long term for older mice, means the activity of ovaries regarding follicular growth, ovulation and CL formation is continue in spite of advanced age. Many experimental work reported that the administration of vitamins maintain performance of the female reproductive system (Bendich and Langseth, 1989; Bayer, 1994).

From the same table, administration of vitamin E for 28 weeks has the best result for diameter of CL when compared to treated and control groups. In general, the administration of vitamin E enhances ovarian performance, and enlarges CL particularly. Although, aged females have limited activity even after hormones therapy (Einspanier, 1997).

After administration of various antioxidants used in this study for 7, 14 and28 weeks, highest number of CL were recorded for hormonally stimulated-mice than natural cycle-mice as presented in figures (22-1, 22-2 and 22-3) respectively.

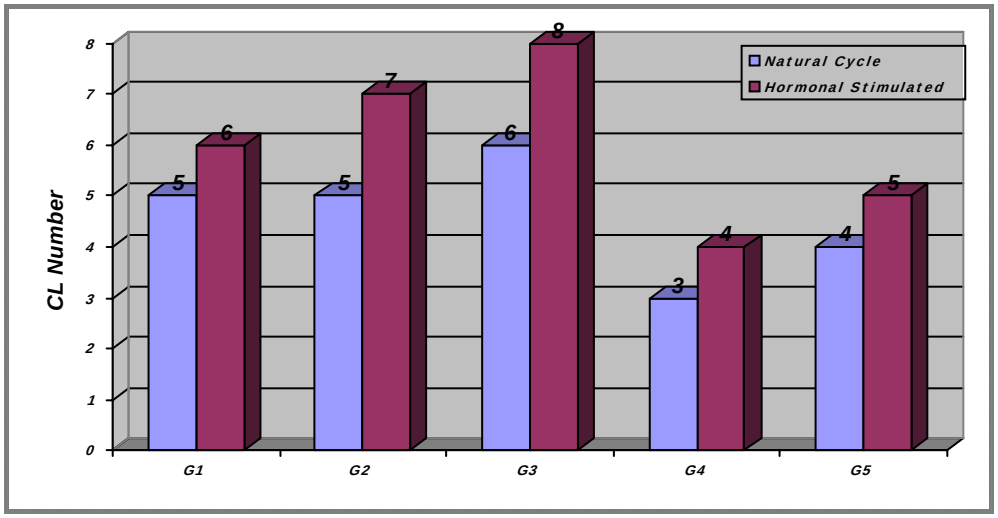

Figure 22-1: 7 weeks of treatment.

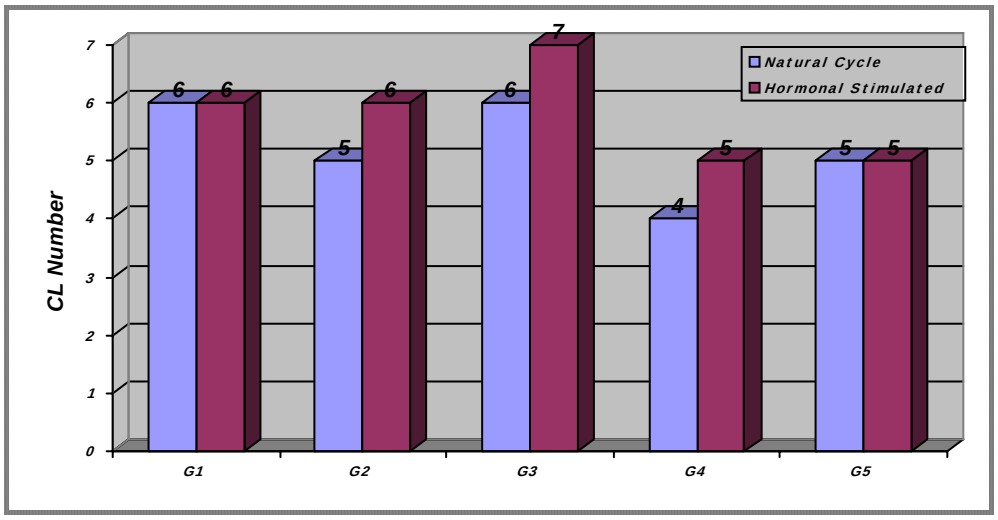

Figure 22-2: 14 weeks of treatment.

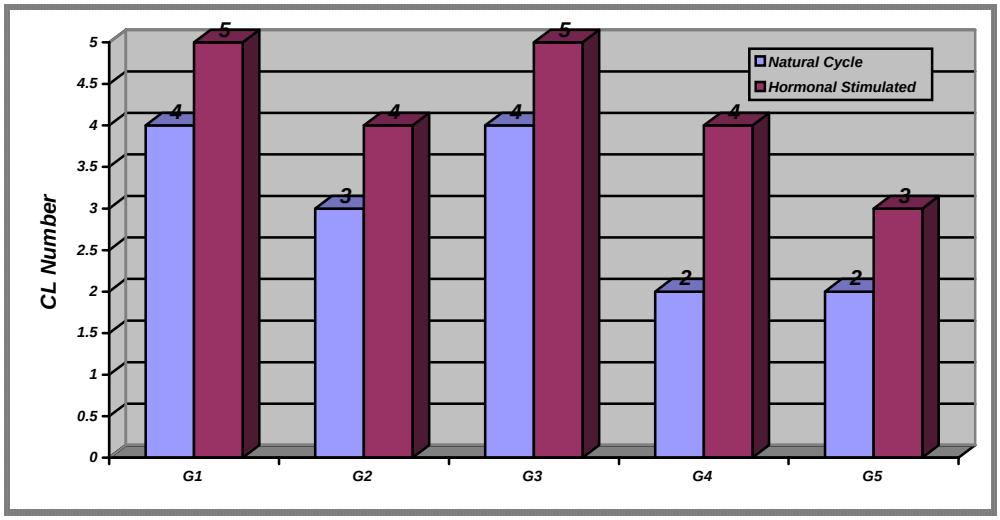

Figure 22-3: 28 weeks of treatment.

Figure 22: Comparison between natural cycle and hormonally stimulated mice in corpus luteum number for all groups administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

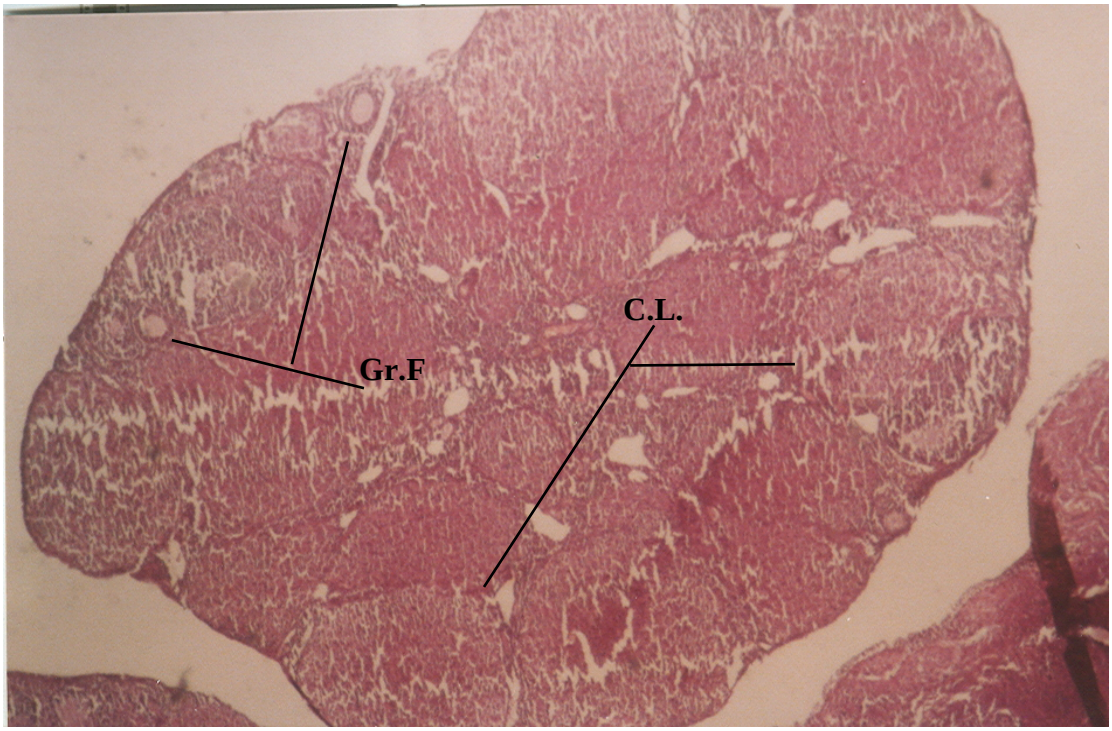

Figure 23: Ovarian longitudinal section for negative control group of natural cycle-mice of 28 weeks periods, stained by Heamatoxylen and Eosin dyes, under magnification power (40X), Gr.F. (Growing Follicle), C.L. (Corpus Luteum).

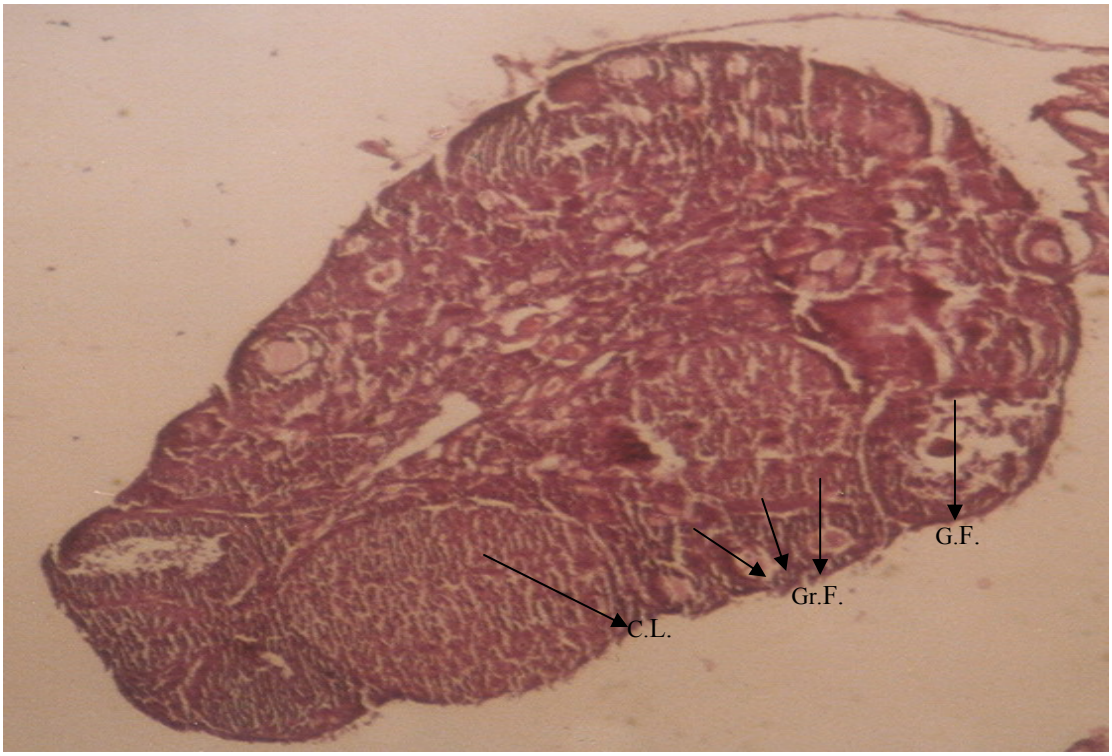

Figure 24: Ovarian longitudinal section for negative control group of natural cycle-mice after 7 weeks C.L. (Corpus Luteum), G.F.(Graafian Follicle), Gr.F.(Growing Follicle), stained by Heamatoxylen and Eosin dyes, under magnification power (40X).

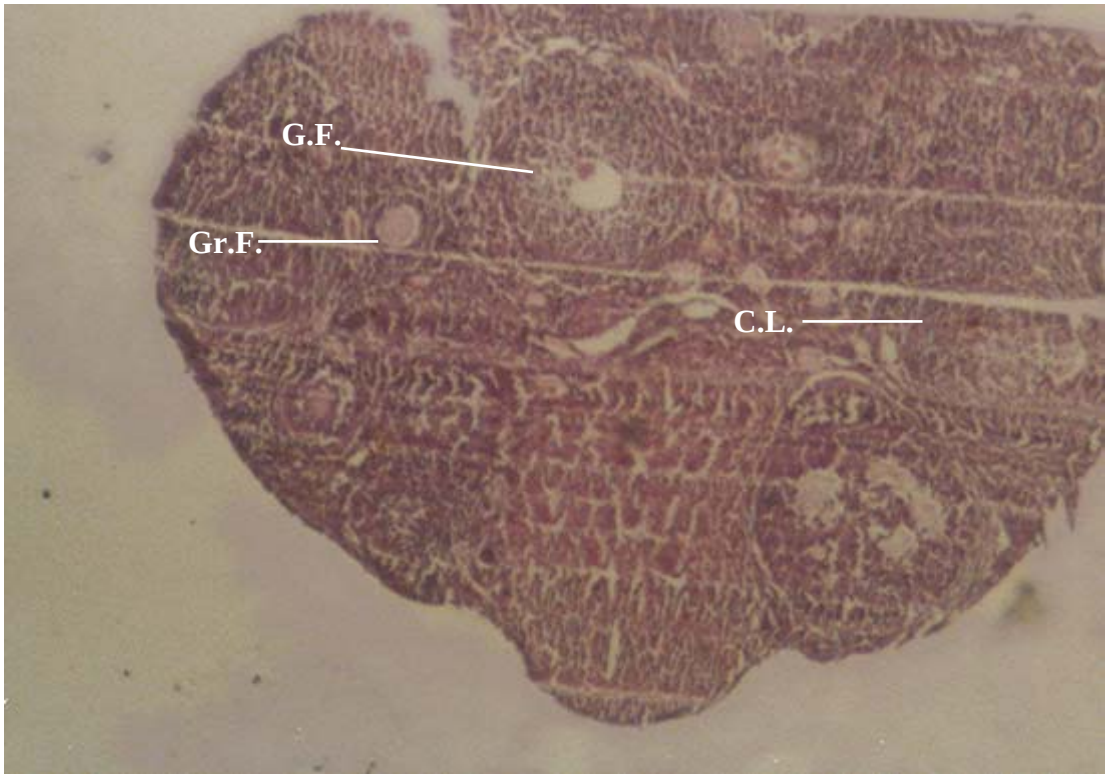

Figure 25 Ovarian longitudinal section of mice treated with vitamin E after 14 weeks hormonally stimulated, stained by Heamatoxylen and Eosin dyes, under magnification power (40X). G.F.(Graufain Follicle), Gr.F. (Growing Follicle), C.L. (Corpus Luteum).

4.9. Total serum cholesterol:

Total serum cholesterol (TSC) was significantly ($P<0.05$) reduced for natural cycle-mice administrated vitamins A+E for 7 and 28 weeks as compared to other treated and control groups. On the other hand, administration of sunflower oil for 14 weeks has the lowest TSC (Table 15-1). Mixture of antioxidants treated for short- or/and for long-term period of time can reduce the level of TSC, as suggestion done by (Behrman and Aten, 1991) they show long-term administration of antioxidants cause lowering level of cholesterol in blood stream. In addition to that, the natural powerful cholesterol-lowering oil is the sunflower oil (Dorrell, 1981).

Table 15: Level of total serum cholesterol from mice administered vitamins A, E and A+E throughout different treatment periods, measured in mg/dl.

15.1. Natural cycle group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	Negative control
7	198.3 ±2.86	187.2 ±2.75	127.2 ±2.86 †	143.7 ±2.53	198 ±0.09
14	85.9 ±2.88	78.9 ±2.89	85.7 ±2.66	62.06 ±2.88 #	200 ±0.76
28	109.8 ±2.76	115.1 ±2.86	105.2 ±2.76	101.4 ±2.43 #	213 ±0.01

Number of mice for each group (6), Values: Mean ± SEM, cholesterol level: (mg/dl) Significant level: P<0.05, †: significant differences with all groups. #: significant differences with negative groups

15.2. Hormonally stimulated group

Group / Weeks	Vitamin A	Vitamin E	Vitamins A+E	Positive control	negative control
7	78.9 ±2.23	189.8 ±2.3	107.7 ±2.54	66.4 * ±2.53	103.03 ±0.89
14	61.01 ±2.4	87.02 ±2.8	79.56 ±2.91	47.8 † 2.56	198.04 ±0.97
28	115.05 ±2.5	113.3 2.6	103.5 # ±2.99	150.1 ±2.66	208.04 ±0.66

Number of mice for each group (6), Values: Mean ± SEM, cholesterol level: (mg/dl) Significant level: P<0.05, *: significant differences with all groups but vitamin A, †: significant differences with all groups #: significant differences with negative groups.

After 7 and 14 weeks periods of various antioxidants administration, the level of TSC was significantly ($P<0.05$) reduced as compared to all groups of hormonally stimulated-mice. While, after 28 weeks treatment, the lowest level of TSC was recorded for hormonally stimulated-mice administered vitamins A+E (Table 15-2). From the same table, the highest levels of TSC were observed for group of negative control mice. In this study, reduction in TSC level recorded for treated and control group. However, the most reduction recorded for the group treated with a mixture of antioxidants (vitamins A+E) with the edible oil (sunflower oil) the most powerful oil falling the TSC level. (Argonz *et al.,* 1950; Sinatra and Demarco, 1995) suggested that antioxidants have a synergism activity with sex hormones to enhance fertility. In this study it was certified that antioxidants with sex hormones injected to female may have the synergism effects on lowering TSC also.

After 7 and 14 weeks periods of administration of antioxidants, levels of TSC for groups of natural cycle-mice are higher than the levels of TSC for groups of hormonally stimulated-mice, except the group of mice administrated vitamin E (Figures 26-1 and 26-2). It was indicated that hormonal injection to females has no significant changes on the level of TSC (Einspanier, 1997). Meanwhile, after 28 weeks period of administration of antioxidants administration of vitamin A for natural cycle-mice reduces the level of TSC more than hormonally stimulated-mice (Figure 26-3). Long-term treatment with vitamin A has accumulatively effects to regulate the TSC level in aged female (Garry *et al.,* 1987; Zuccotti, 1998).

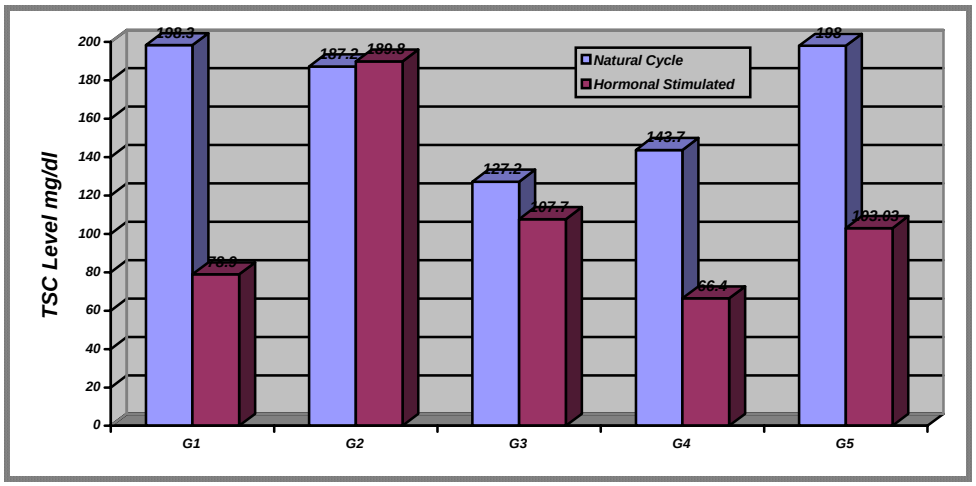

Figure 26-1: 7 weeks of treatment.

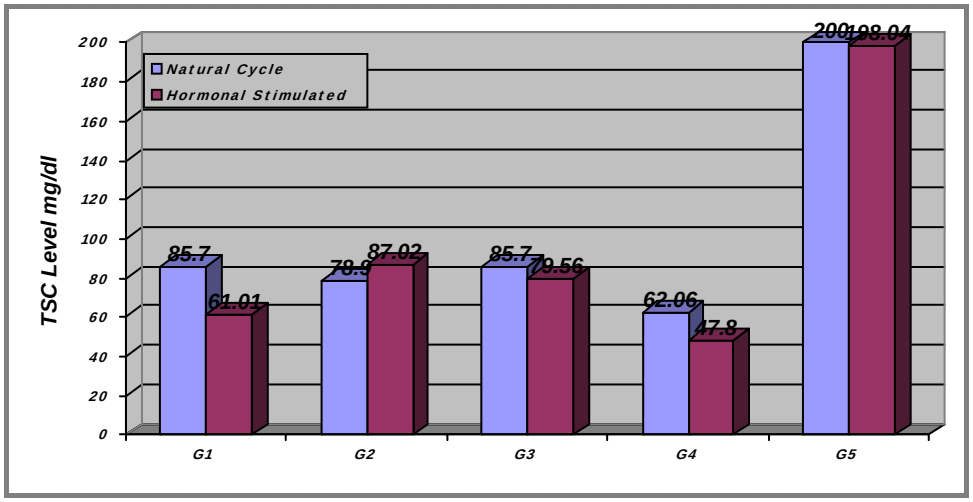

Figure 26-2 after 14 weeks of treatment.

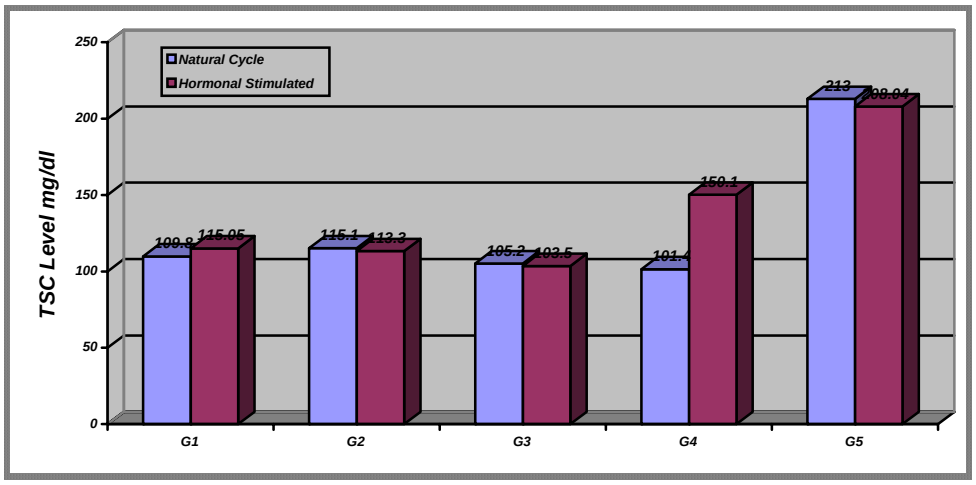

Figure 26-3: after 28 weeks of treatment.

Figure 26:Comparison between natural cycle- and hormonally stimulated-mice in serum cholesterol level for all groups administrated vitamin A, vitamin E, vitamins A+E, positive and negative control; G1,G2, G3, G4 and G5 respectively.

Conclusions

1. Administration of vitamins A and E as antioxidants, enhance the body weight and correlate with period of administration, especially for 14 weeks periods.
2. Administration of vitamins A and E as antioxidants improves the activity of uterine horns and ovaries, and their fertility potential for longer periods. Also, outcomes of *in vivo* and *in vitro* fertilization are enhanced.
3. Administration of vitamins A and E as antioxidants results in lowering the level of total serum cholesterol for all treated periods, but the best with 14 weeks treatment.
4. Outcomes of super ovulation are significantly improved when vitamins A+E are used for 14 and 28 weeks of treatment.
5. Long-term administration of sunflower oil as antioxidant has limited positive effects on fertility performance. However, it has powerful activity for reducing level of total serum cholesterol.

Recommendations

1. Further molecular and biochemical studies are recommended to investigate the mechanisms of vitamin actions related to the fertility, growth regulations and lowering level of cholesterol.
2. Further endocrinological study, on the effect of vitamins A and E on growth and sex hormones and their correlation with the total serum cholesterol.
3. Further study on ovarian reserve in the aged female in relation to the daily intake of these antioxidants.
4. Applied study on fertility of human being administered vitamins as antioxidants according to the recorded benefits on animals.

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الخلاصة

مضادات الأكسدة وهي مركبات كيميائية تؤخر بداية تفاعل أكسدة الشحوم في النظام الغذائي أو تبطئ معدله. مضادات الأكسدة تعتبر واحدة من أفضل عناصر الحماية للجسم من المؤثرات الجانبية للجذور الحرة وتدعم عوامل النمو؛ تحسين مستوى الخصوبة و تقلل مستوى الكولسترول في مصل الدم. ولهذا وفي هذه الدراسة خططت إلى التحري عن تأثيرات التجريع الفموي (الذي لايتجاوز المسموح به يوميا من مختلف مضادات الأكسدة المتضمنة الفيتامينات المذابة بالدهون فيتامينات أ, هـ وزيت زهرة الشمس) على كل في وزن الجسم, ونتائج الإخصاب الخارجي و الداخلي و مستوى الكولسترول في مصل الدم والتغيرات النسيجية لكل من قرون الرحم والمبايض.

مئة و ثمانون أنثى فار سليمة قسمت إلى ثلاث مجاميع رئيسية بالنسبة إلى مدة تجريع مضادات الأكسدة متضمنة 7, 14 و28 أسبوع, كل مجموعة رئيسية تقسم إلى خمسة مجاميع فرعية بالنسبة إلى نوع مضاد الأكسدة متضمن فيتامين أ, فيتامين هـ , فيتامينات أ+هـ, مجموعة سيطرة ايجابية (زيت زهرة الشمس) ومجموعة سيطرة سلبية.

نتائج هذه الدراسة لخصت بالتالي:

1 . وزن الجسم يزداد للفئران المعاملة بفيتامين هـ (7, 14 و 28) أسبوع, وهذه المجموعة تشاطر نفس الارتفاع لفترات المعاملة بفيتامينات أ+هـ(14) أسبوع.

2. الفئران المحفزة هرمونيا لها أعلى وزن مطلق للأعضاء التناسلية من الفئران طبيعية دورة الاباضة لفترات المعاملة الثلاثة,تجريع مختلف الفيتامينات له أفضل نتائج أوزان مطلقة للأعضاء من مجاميع السيطرة السلبية, بينما النتائج المسجلة للأوزان المطلقة لكل من المبايض و قرون الرحم للمجموعة المعاملة بفيتامينات أ+هـ لفترة 14 أسبوع هي الأثقل وزنا.

3. المجاميع المعاملة مع مضادات الأكسدة المختلفة سجلت أفضل نتائج لأوزان الحوامل ووزن الجريبة من مجموعة السيطرة السلبية لفترات المعاملة الثلاثة, في حين أحسن نتيجة سجلت لنتائج الإخصاب الداخلي للفئران المعاملة بفيتامينات أ+هـ و فيتامين هـ لمدة 14 أسبوع.

4. مجموعة الفئران المعاملة بفيتامينات أ+هـ أظهرت أفضل نتائج إخصاب خارجي خصوصا بإجمالي البويضات المجموعة من نفس الفئران معاملة لفترة 14 أسبوع ولكن أفضل نسبة مئوية للتنضيج الخارجي و الإخصاب الخارجي سجلت لمجموعة الفئران المجرعة فيتامينات أ+هـ لسبعة أسابيع في حين اقل نسبة مئوية لتطور جنيني غير طبيعي سجل لمجموعة الفئران المجرعة فيتامين هـ 14 أسبوع.
5. الدراسة النسيجية أظهرت ذلك بان قرون الرحم و المبايض لمجاميع التحفيز الهرموني معاملة بمضادات الأكسدة لها أفضل صفات لكلا المقاطع الطولية من مجاميع السيطرة للمحفزة هرمونيا ولطبيعية دورة الاباضة. الصفات الرحمية تتضمن سمك الطبقة الظهارية و قطر قرن الرحم والغدد الرحمية. و بالاظافة لقد سجلت ذلك بان الفئران المحفزة هرمونيا المعاملة بفيتامين هـ و فيتامينات أ+هـ للفترات المعاملة الثلاثة هي الأفضل بكل تلك الصفات. و أكثر من ذلك أفضل نتائج بالنسبة للأعداد و الأقطار لكل من الحويصلات النامية و حويصلات كريفيان و الجسم الأصفر سجلت للفئران المحفزة هرمونيا المعاملة بفيتامين أ و فيتامينات أ+هـ(7 و 14) أسبوع, بينما التجريع بفيتامين هـ يملك أفضل النتائج بالنسبة لأقطار وأعداد الجسيمات الصفراء للمجاميع المعاملة الثلاثة.
6. المعاملة لفترات طويلة بمضادات الأكسدة تقلل مستوى الكولسترول في مصل الدم مقارنتا بمجاميع السيطرة السلبية واقل نسبة للكولسترول في مصل الدم لوحظت لمجموعة السيطرة الايجابية بعد 14 أسبوع من المعاملة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

تأثيرات بعض الفيتامينات الذائبة بالدهون و زيت زهرة الشمس على مستوى الإخصاب و مستوى الكولسترول في مصل الدم في إناث الفئران

أطروحة مقدمة إلى كلية العلوم جامعة النهرين كجزء من متطلبات الحصول على درجة الماجستير للعلوم في التقانة الإحيائية

من قبل
نياف نضال كاظم الشمري
بكالوريوس ٢٠٠٢
جامعة النهرين

د. خلود وهيب السامرائي

د. محمد باقر محمد رشاد فخر الدين

كانون الاول, ٢٠٠٥

ذوالقعدة, ١٤٢٦